▼ Review Article

Acute Toxicity of Local Anesthetics: Underlying Pharmacokinetic and Pharmacodynamic Concepts

Laurence E. Mather, Ph.D., Susan E. Copeland, MVetClinStud, and Leigh A. Ladd, B.VSc.

The risk of accidental intravascular injection and consequent acute toxicity is ever-present with most neural blockade techniques. The severity of cardiovascular and central nervous system (respectively, CVS and CNS) toxicity is directly related to the local anesthetic potency, dose, and rate of administration. Nonetheless, although the anesthetic potency of ropivacaine and levobupivacaine is similar to that of bupivacaine, at usual clinical doses, ropivacaine and levobupivacaine are less likely than bupivacaine to cause convulsions or lethal dysrhythmias. Signs of CNS stimulation, ranging from tremors to convulsions and perhaps cardiac dysrhythmias, can be described in terms of a chaos-derived state change in which the local anesthetic appears to act as an initiator. Both CNS and CVS effects are rather poorly correlated with arterial drug concentrations but better correlated with concentrations in the respective regional venous drainage. Lung uptake reduces the maximum drug concentration by ~40%. Prolonging intravenous administration from 1 to 3 minutes results in a similar decrease in maximum concentration. This is an underlying tenet of dose fractionation, but the main advantage of dose fractionation is that the anesthesiologist is able to cease administration with less of the dose given if signs or symptoms of toxicity occur. Overall, it appears that the gains in safety from ropivacaine and levobupivacaine are due more to favorable pharmacodynamic enantioselectivity than to pharmacokinetic factors. This essay presents some pharmacokinetic aspects relevant to acute toxicity of local anesthetics, mainly using data from the authors' studies in a sheep model of simulated accidental intravenous administration. *Reg Anesth Pain Med 2005;30:553-566.*

The matter of maximum recommended doses of local anesthetics was recently reviewed by Rosenberg et al.[1] They concluded that the present approach, which is based on individual drugs, was not soundly evidence based and that new recommendations should be based on specific nerve blocks with modifications according to patient characteristics to account for individual differences in pharmacokinetics of systemically absorbed drug and the concomitant risk of systemic toxic side effects.

See Editorial page 513

From the Department of Anaesthesia and Pain Management, University of Sydney at Royal North Shore Hospital, Sydney NSW, Australia.

Accepted for publication July 8, 2005.

Reprints: Laurence E. Mather, Ph.D., Department of Anaesthesia and Pain Management, University of Sydney at Royal North Shore Hospital, Sydney NSW 2065 Australia. E-mail: lmather@med.usyd.edu.au

1098-7339/05/3006-0009$30.00/0
doi:10.1016/j.rapm.2005.07.186

Implicit in any maximum recommended dose (or minimum effective dose) are the dual requirements for the dose to be large enough to produce the desired effect, in this case neural blockade, but small enough to reasonably preclude side effects, in this case central nervous system (CNS) and cardiovascular system (CVS) toxicity caused by systemically absorbed local anesthetic. These side effects, as pointed out in Rosenberg et al.'s review,[1] are easier to understand when the systemic pharmacokinetics of the drugs, and (patho-)physiologic factors that affect these pharmacokinetics, are known. However, a more fearsome side effect of local anesthetics is acute toxicity caused by accidental intravascular administration, occasionally intra-arterial but more commonly intravenous. In such a situation, even these revised dosage recommendations may be problematic because the local anesthetic dose required for most nerve blocks could produce acute toxicity if injected intravascularly. This topic of intravascular accidents and resultant acute toxic-

ity was not included in the previous review,[1] but it has been reviewed recently, principally from the clinical viewpoints of incidence, prevention, and resuscitation.[2-4] However, it would appear that a pharmacokinetic-pharmacodynamic viewpoint of acute toxicity has not previously been published.

Therefore, the purpose here was to add to the information provided in the previous reviews by describing some of the pharmacokinetic and pharmacodynamic research on acute local anesthetic toxicity from accidental intravascular administration. To do this, the authors have mainly drawn on their own laboratory studies of systemic toxicity from simulated clinical accidents with local anesthetics in large animals (sheep). These studies were mainly concerned with preclinical evaluation of the newer long-acting local anesthetics and were intended to complement more limited studies in humans. Some of these data have been previously published but are re-presented here in new form. All of the authors' studies were approved by appropriate ethics review panels.

Human Problems to Animal Research

Local Anesthetic Toxicity and Its Investigation

Systemic toxicity from local anesthetics is relatively rare, and its incidence appears to be decreasing, corresponding, it would seem, to the widespread introduction of procedural safety steps[2,3] aided by safer local anesthetics.[3-5] Mulroy[2] described an incidence of approximately 12/100,000 epidural anesthetics and 200/100,000 brachial plexus blocks. More recent data from 4,291,925 deliveries of anesthesia in the operating rooms of Japanese Society of Anesthesiologists Certified Training Hospitals indicated that the frequency of local anesthetic toxicity, as distinct from other critical incidents and complications of neural blockade, was approximately 1.17/100,000 anesthetic deliveries, with a fatality rate of 0.023/100,000.[6] Despite its rarity, local anesthetic toxicity can be catastrophic to the individual when it occurs.

Although many anesthesiologists may occasionally see mild manifestations of toxicity, most never encounter serious intoxication. Nonetheless, it is probable that many more "near misses" occur undetected. Indeed, it was recently stated that ". . . it is not a question of 'if' an intravascular injection will occur, [it is] just a question of 'when'. . ."[7]

The Need for Large-Animal "Models"

Most data documenting acute local anesthetic toxicity in humans are gathered in retrospect, essentially opportunistically, or from small cohort prospective studies with low power, so that levels of evidence for most human data will never be high. For ethical reasons, human subjects can be given only mildly toxic doses when local anesthetics are deliberately administered intravenously for research, typically to the subjective onset of CNS symptoms.[8-12] Information about more serious toxicity must therefore be derived either from clinical circumstances in which the objectives are preservation of life and well-being, rather than acquisition of scientific data, or from laboratory animal "models." Therefore, human studies can only be a "blunt instrument" for such investigations. Laboratory rodent and isolated tissue models are widely used and are especially useful in elucidating mechanisms and for between-drug comparisons. These are generally "sharp, but limited, instruments" because of the restricted types of data that can be obtained or because of their destructive nature and/or isolation of tissues from their normal milieu. On the other hand, anatomically and physiologically sound studies in large experimental animal models can be a "very sharp instrument" to observe extensive whole-body pharmacokinetics and pharmacodynamics in a context quite similar to patient treatment.

Research data from various large-animal models used to investigate local anesthetic intoxication has been recently compiled by Groban.[13] The present article provides more comprehensive data from just one of these models: the adult female sheep preparation used by the authors. The major differences between this and other models, apart from species or gender, are that both CNS and CVS data are collected concurrently from discrete doses in conscious, preprepared, closed-chested, chronically maintained animals, after their recovery from surgical preparation. This model closely resembles the situation in which local anesthetic is accidentally administered intravascularly to human patients undergoing neural blockade. It has allowed pharmacokinetic and dose-response relationship data to be acquired for systemic toxic effects ranging from slight to lethal in essentially unmedicated "normal" subjects.[14,15] Moreover, because intravenous local anesthetic administration provokes both direct CVS and indirect (CNS-mediated) effects (at least in conscious subjects), the direct and indirect effects of these agents on the brain and heart can be studied separately by administration of the local anesthetics directly into the respective organ's arterial blood supply under the same experimental conditions as those used for simulated intravenous accidents.[14-17]

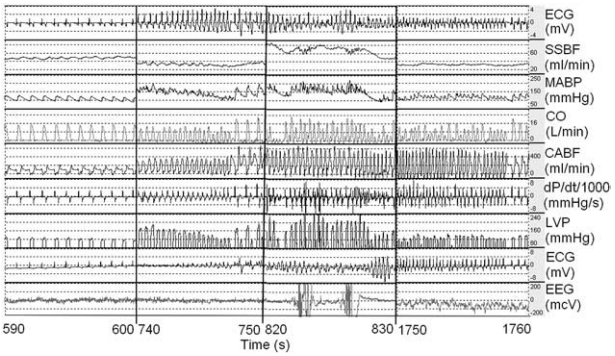

Fig 1. Physiographic data showing the time course of bupivacaine-induced CNS and CVS intoxication in a previously prepared conscious adult sheep. Data were acquired digitally at 256 Hz for 10 minutes before, during 3 minutes of intravenous infusion of 100 mg bupivacaine HCl in 30 mL saline, and for 60 minutes afterwards. Sections: 590 to 600 seconds (immediately before drug infusion), 740 to 750 seconds (30 seconds before end of infusion), 820 to 830 seconds (40 seconds after ceasing infusion), 1,750 to 1,760 seconds (approximately 20 minutes after starting infusion). Traces in order from top: craniocaudal ECG, sagittal sinus blood flow (SSBF), mean arterial blood pressure (MABP), cardiac output (CO), left coronary artery blood flow (CABF), left ventricular dP/dt_{max}, left ventricular pressure (LVP), caudoventral ECG, and total power (1-32 Hz) in the cortical (dural) EEG.

A Case of Acute Bupivacaine Toxicity

Figure 1 shows a time course of effects during serious, but nonfatal, toxicity from bupivacaine HCl (100 mg in 30 mL saline, infused intravenously over 3 minutes) in a conscious sheep. The panels show electrocardiogram (ECG) and cortical electroencephalogram (EEG) traces, mean arterial blood pressure, left ventricular pressure and its first derivative (dP/dt_{max}, an index of myocardial contractility), pulmonary artery blood flow (pulsatile cardiac output), left coronary artery blood flow, and sagittal sinus blood flow; data were acquired before (0 to 10 minutes), during (10-13 minutes), and after (13-70 minutes) the infusion. Relevant blood samples were collected to correlate circulating bupivacaine concentrations, shown in Figure 2, with the pharmacodynamic effects. The features of bupivacaine intoxication shown here would also be expected to occur in a human patient after accidental intravenous injection of ~2 mg/kg (i.e., a dose similar to the present maximum recommended dose of bupivacaine), administered over a time period consistent with dose fractionation.

Acute hemodynamic changes began during the first 2 minutes with myocardial depression, revealed by decreased dP/dt_{max}, followed soon after, and coinciding with the onset of CNS stimulation, by an abrupt and marked increase in myocardial contractility, accompanied by increases in mean arterial blood pressure, heart rate, and cardiac output. By the time convulsions occurred (shown by bursts of EEG overactivity), disturbed cardiac conduction, with widened QRS complexes and ventricular dysrhythmias, was also present. Without treatment, the cardiovascular and CNS changes lasted for ~20 minutes, when there was a sudden return to normal heart rate and rhythm; the EEG activity returned to normal ~40 minutes later.

A peak arterial blood (total) bupivacaine concentration of ~8 μg/mL occurred, as expected, at the end of the 3-minute infusion; unbound drug blood concentrations (see later) were not measured. The arterial blood bupivacaine concentration was ~7 μg/mL at ~2 minutes, when both CNS and CVS toxicity began (Fig 2). Bupivacaine concentrations in coronary sinus and sagittal sinus blood (venous drainage of the myocardium and brain) reached maxima of ~4 and ~2 μg/mL a few minutes later, corresponding to the times of peak concentrations of drug in those tissues. By the time that all effects had dissipated, blood bupivacaine concentrations at all 3 sampling sites had decreased to ~1 μg/mL.

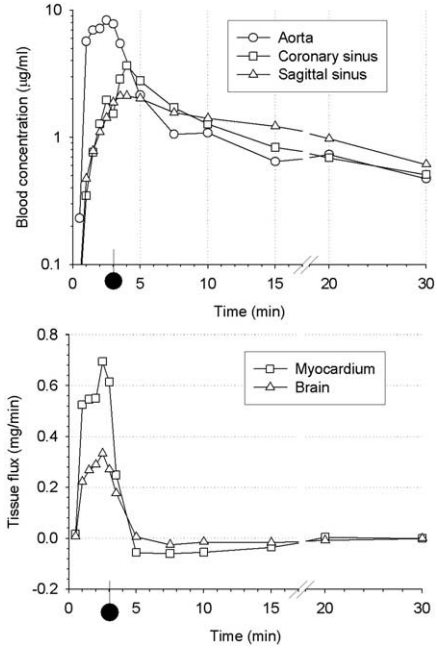

Fig 2. Upper panel: bupivacaine concentrations (as racemate) in arterial, coronary sinus, and sagittal sinus of the subject shown in Figure 4. Lower panel: net flux of bupivacaine between blood and myocardium and between blood and brain, as determined from the product of the relevant respective drug arteriovenous concentration gradient and blood flow. Time 0 marks the start and the solid circle marks the end of the drug infusion period.

Figure 2 also shows the tissue (myocardium and brain) net flux; this is the instantaneous net rate of drug exchange between blood and tissues serially calculated from the respective products of the arteriovenous drug concentration differences and regional blood flows. Net flux is positive when there is net transfer from blood to tissue, zero when the blood and tissue concentrations are equal, and negative when the net transfer is from tissue back to blood. The maximum influx occurred at the end of the infusion, thereafter quickly becoming negative and slowly returning toward zero as the arterial blood drug concentrations continuously decreased because of dilution (tissue uptake elsewhere) and clearance (metabolism mainly and excretion). Thus, the initial loading (uptake) of vital tissues with bupivacaine was very rapid, and unloading commenced soon after and continued, albeit slowly, for a very long time. This pattern is consistent with facile diffusion of this lipophilic drug and the high perfusion of these tissues.

The acute hemodynamic changes (Fig 1) are the net resultant of direct and indirect CVS effects, the latter mediated mainly via the CNS.[18] This complex interaction between the CNS and CVS is characteristic of the whole-body response to acute toxicity from large intravenous doses of local anesthetics in conscious subjects. To study the cardiac effects of local anesthetics without the influence of CNS, various investigators have used site-directed coronary arterial administration in large animals, principally acutely prepared pigs[19,20] and dogs[21] and chronically prepared conscious sheep.[16] The results of these in vivo studies are essentially similar to those of ex vivo studies using isolated heart tissue.[22] Overall, local anesthetics cause dose-related direct myocardial depression with potency essentially proportional to potency for neural blockade, and they also disturb cardiac conduction, shown primarily by QRS complex widening and dysrhythmias, with death caused by pump failure and/or malignant dysrhythmias.[16,19,20]

Information about indirect CNS-mediated effects of blood-borne local anesthetics on CVS has been provided from site-directed carotid arterial infusion in chronically prepared conscious sheep.[17] These experiments have shown that CNS effects (convulsions) of blood-borne local anesthetics result in myocardial stimulation (increased left ventricular dP/dt_{max}) but not necessarily cardiac dysrhythmias, apparently unlike introduction of drug directly into the brain in which cardiac neurovascular control may become deranged and in which cardiac dysrhythmias may occur.[23-25]

Table 1. Intrinsic Whole-Body Toxicity of Some Important Local Anesthetic Agents as Determined by the Median Lethal Dose After Intravenous Injection in the Mouse

	Mather[28]	Aberg[26]	Akerman et al[29]
Prilocaine*	37.8 ± 1.2		
Lidocaine	28.8 ± 1.4		18.8 ± 2.5
Mepivacaine*	27.8 ± 1.1	35 ± 3.0	
R(−)-Mepivacaine		32 ± 2.5	
S(+)-Mepivacaine		34 ± 3.5	
Ropivacaine			11.0 ± 0.2
Bupivacaine*	5.2 ± 0.2	7.3 ± 1.0	7.9 ± 0.4
R(+)-Bupivacaine		7.9 ± 1.0	
S(−)-Bupivacaine†		9.6 ± 1.0	

NOTE. Dose is given in mg/kg of local anesthetic base; error is the standard error of the mean.
*Clinically-used racemate.
†Now also known as levobupivacaine.

Local Anesthetic Chemistry and Pharmacodynamic "Potency"

The potency for CNS stimulatory (proconvulsant) effects of local anesthetics parallels their potency for neural blockade but with substantial differences between the enantiomers of bupivacaine.[17,26] This is not surprising because of the conservation of genetic coding among the superfamily of voltage-gated sodium channels in various tissues.[27] Because, as mentioned earlier, direct myocardial depression also is proportional to potency for neural blockade, it is also not surprising that over a wide array of local anesthetics with different chemical structures, local anesthetic potency is also proportionally associated with lethality, judged by intravenous median lethal dose values in laboratory rodents[26,28,29] (Table 1). There are appreciable differences between enantiomers of certain chiral local anesthetics: these principally relate to different affinities for ion channel receptors.[30] Nevertheless, despite preservation of this rank order in a variety of experimental designs, the mechanisms of lethal intoxication appear to differ among local anesthetics with different structures in both conscious and anesthetized subjects.[31,32] On one hand, intravenous bupivacaine is more likely to produce fatality by sudden onset of lethal dysrhythmias; on the other, intravenous lidocaine is more likely to produce progressive contractile failure. Intravenous ropivacaine and levobupivacaine have produced fatalities by either mechanism, and it is not yet clear what makes one outcome more likely than the other in individual cases. In addition, differences between local anesthetics in the cardiac contributions of direct and indirect toxic effects remain perplexing.

When infused directly into the left coronary ar-

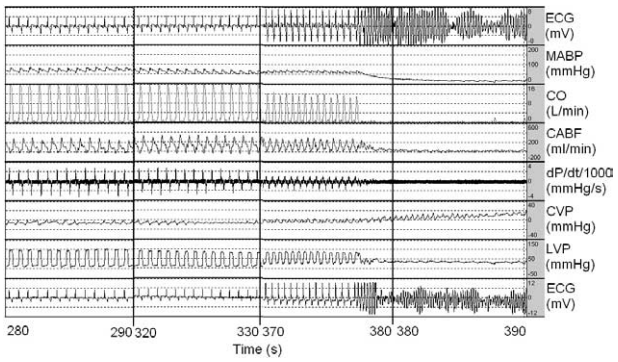

Fig 3. Cardiac effects of lidocaine infused into the left coronary arteries of a previously prepared conscious adult sheep. A dose of 40 mg/min was infused from time = 300 seconds. Data acquisition methods and abbreviations are as described for Figure 1. Sections: 290 to 300 seconds (immediately before drug infusion), 320 to 330 seconds, and 370 to 390 seconds. The sheep was prepared according to our normal procedures. Death occurred by sudden onset of dysrhythmias similar to those caused by bupivacaine immediately followed by cardiovascular collapse.

teries of conscious sheep[16] and anesthetized pigs,[20] lethal ventricular fibrillation occurred with bupivacaine, levobupivacaine, and ropivacaine. Whereas there were no significant differences between drugs in lethal doses when infused as a small discrete dose into coronary arteries of conscious sheep,[16] differences between drugs were found when infused as cumulative doses in anesthetized pigs such that the rank order of lethal potency was bupivacaine > levobupivacaine ≥ ropivacaine.[20] Furthermore, as shown in Figure 3, lethal dysrhythmias and cardiovascular collapse can occur also with intracoronary infusion of lidocaine in both conscious[16] and anesthetized subjects.[19,20] Such examples show clearly that sudden-onset cardiac collapse from a local anesthetic need not be related to concurrent CNS intoxication.

Pharmacokinetic and Pharmacodynamic Interpretations of "Blood Drug Concentrations"

It can be seen from Figures 1 and 2 that a simple monotonic drug dose or blood drug concentration-response relationship cannot be shown for the hemodynamic changes in vivo, as can be normally shown in preparations ex vivo. The data suggest that a bupivacaine arterial blood concentration of ~7 μg/mL is both convulsant and dysrhythmogenic, but the convulsions and dysrhythmias both vanish when the concentration has decreased to ~1 μg/mL. These observations raise a fundamental question: what is the "toxic concentration?" The situation becomes especially complicated with a racemic drug such as bupivacaine because of differential toxicity and pharmacokinetics of the enantiomers. Both systemic (or whole body) and regional pharmacokinetics are relevant in this context, and they are complementary.

Systemic pharmacokinetics are time-averaged hypothetical properties derived from curve-fitting drug concentration-time data sets: their relevance to systemic absorption of local anesthetics was reviewed by Rosenberg et al.[1] After intravenous injection, the regional pharmacokinetics are especially important: these are empirical properties derived from products of the respective concurrent regional blood flows and arteriovenous drug concentration gradients.[33] For local anesthetic toxicity, the rates of drug uptake (influx) into the brain and heart are the most relevant. However, because of the requirement to obtain afferent and efferent blood drug concentrations and regional blood flows, regional pharmacokinetics are virtually impossible to obtain, except in large experimental animals. Fluxes so-measured in vivo are net fluxes because they are the difference between influx and efflux.

In addition to the previously described considerations, general anesthesia is well documented to alter pharmacokinetics[34] and drug effects and thus may affect interpretation of research data from various investigations of local anesthetic intoxication. Regardless of the pharmacokinetic approaches used, the potential impact of concomitant general anesthesia on various research models must be taken into consideration.[35,36]

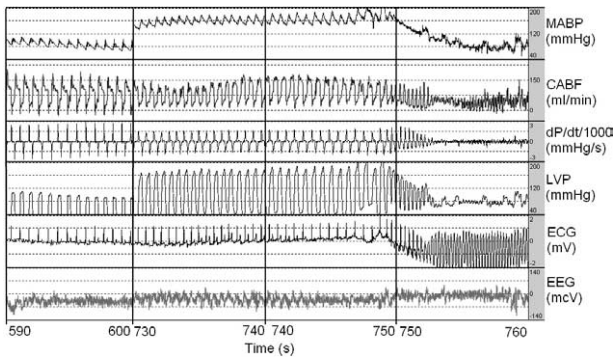

Fig 4. Physiological effects of intravenous infusion of ropivacaine HCl (150 mg over 3 minutes) in an adult sheep. Data acquisition methods and abbreviations are as described for Figure 1. Convulsions commenced at 2.1 minutes (at 726 seconds), and death occurred abruptly at 2.6 minutes (at 756 seconds) after commencement of infusion.

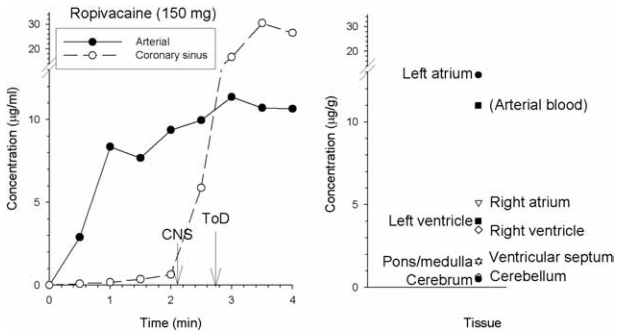

Fig 5. Arterial and coronary sinus blood, heart, and brain concentrations of ropivacaine after intravenous infusion of 150 mg over 3 minutes in the study shown in Figure 4. Blood was sampled until 4 minutes; the massive increase in coronary sinus blood ropivacaine concentration post mortem does not reflect the tissue concentrations and presumably results from a combination of factors, including collection of infused drug solution with post mortem vascular pressure changes.

Anatomical and Physiological Interpretation of "Blood Drug Concentrations"

An example showing sudden death of a sheep after intravenous infusion with ropivacaine further illustrates the complicated interaction between pharmacokinetics and pharmacodynamics and difficulties in describing "toxic blood concentrations" (Figs 4 and 5). The point of measuring circulating blood (or plasma or serum) drug concentrations is that, after initial distribution, they appear to be in pseudo-equilibrium with the drug concentrations in tissues[37,38] so that any systemic response to the drug would be more predictable from, and related to, blood drug concentrations than dose alone. But soon after an intravenous administration, before pseudo-equilibrium has been attained, even highly perfused tissues do not necessarily behave as a "homogeneous compartment"; thus, blood drug concentrations may be only roughly related to drug effect, especially if profound physiological disturbances are also occurring. However, the total-body burden (or load) of drug will also be an implicit determinant of the drug effect because it influences the magnitude of the relevant regional net flux and the time over which drug concentrations remain in the "active" region. Acute toxic effects from intravenous administration are thereby likely to be more severe, but shorter lived, than if caused by relentless systemic absorption from perineural injection when the dose and absorption rate exceed the capacity of the body for drug clearance.

Pharmacodynamic interpretation of "blood drug concentrations" is influenced by the site, time after drug administration, and conditions under which the blood samples are obtained. Whereas arterial drug concentrations are essentially the same wherever sampled, venous concentrations depend on drug solubility and metabolism in the region being drained. However, venous blood samples, usually from an antecubital vein in humans, are commonly analyzed because of their ease of collection, but their concentrations are damped and temporally delayed because of the transit and solubility of the drug in the (forearm) region being drained. The time after drug administration is a "hidden" variable in that it influences different drugs to different extents. Hence, if, as is sometimes done in human and animal studies, comparison between pharmacological tolerability of different drugs is made on the basis of the tolerated blood drug concentration, then the comparison can be skewed by differences between drugs in their rates of tissue uptake and metabolism.

Moreover, both arterial and venous blood drug concentrations are influenced by hemodynamic factors,[37] but the product of blood flow and drug concentration will be constant because of the conservation of matter, with all other things being equal.[33] All of these factors impinge on interpretation of blood drug concentration in relation to effect and clearly cannot be studied systematically in humans. For example, after death, sampled blood (particularly from the heart) may be contaminated by drainage from different sites as vascular pressures fall, including those from blood vessels enriched with injected drug solution (Fig 5). The consequence is that the relationship between blood and tissue drug concentrations and side effects becomes tenuous and can make interpretation unexpectedly hazardous, especially if used for forensic purposes (e.g., to calculate whether a dosage error has been made on the basis of post mortem drug concentration data). Hence, by offering a "calibration factor," systematic laboratory animal studies of regional pharmacokinetics have an important role in understanding human cases of acute intoxication that cannot be studied so invasively.

Dose Dependence of Local Anesthetic Intoxication

Although it is generally held that serious cardiac intoxication from local anesthetics lags temporally behind CNS intoxication, this was not observed in Figure 1. Are there characteristic "toxic" blood drug concentrations, as suggested earlier?

The abrupt onset of convulsions is sometimes the first indication of serious local anesthetic intoxication. The progression of acute CNS toxicity appears, after reaching some ill-defined threshold, to follow

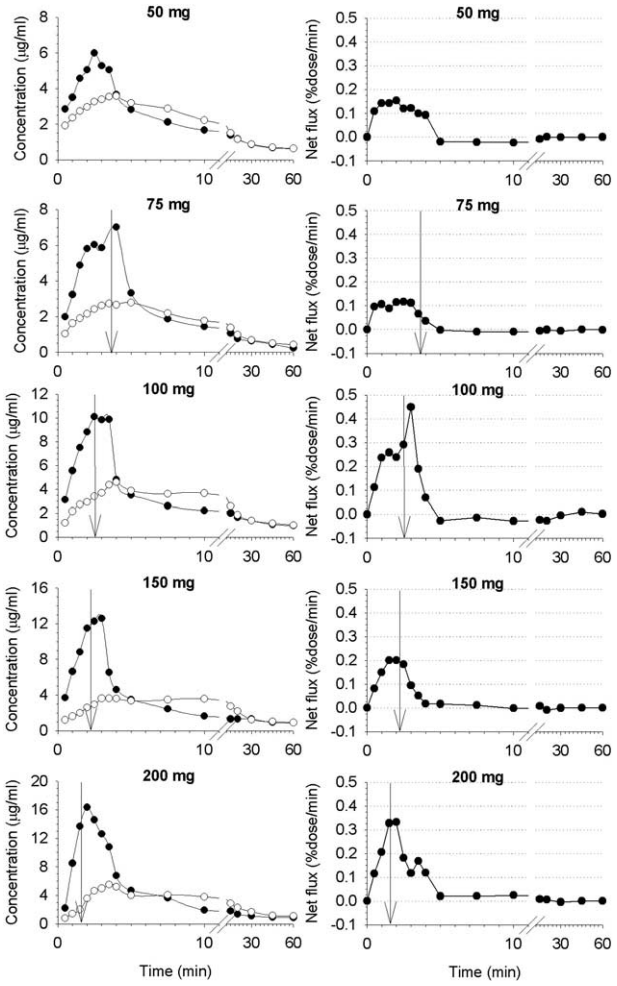

Fig 6. Blood concentration-dose relationships for levobupivacaine after intravenous infusions made over 3 minutes on separate occasions in the same conscious sheep subject. Left panels: arterial (closed circles) and sagittal sinus (open circles) blood levobupivacaine concentrations. Right panels: net flux (as % dose/min; net influx is positive and net efflux is negative) of levobupivacaine across the brain. Arrows indicate the time to onset of convulsions. There was no convulsion from the 50 mg dose.

a chaotic model. As time progresses, the subject constantly reaches new CNS "state" levels that summate temporally to either advance the effect, ultimately leading to convulsions, or extinguish the effects. Although the progression of effects clearly depends to some extent on receptor occupancy, it seems that local anesthetics behave more as a threshold initiator than as an agonist in a simple deterministic manner whereby its degree of receptor occupancy determines the intensity of CNS stimulation.[18]

tk;4Attempts have been made to define the "convulsant arterial blood drug concentration" so that it can be avoided. A significant problem is that this concentration is not a constant and varies with the speed of injection of the drug, among other things. This is illustrated in Figure 6, using an example of increasing doses of levobupivacaine in a sheep, in which the arterial drug concentration at onset of convulsions appears to increase with increasing dose, with a corresponding decrease in the time of onset. Figure 7 shows composite data from a cohort of animals infused intravenously with increasing discrete doses of levobupivacaine and bupivacaine. Convulsions from levobupivacaine appear to begin at a lower arterial blood drug concentration than from bupivacaine at the 75 mg/3 min dose (at which 5/6 convulsed with bupivacaine and 3/7 with levobupivacaine). However, this is partly an artifact of the duration of infusion in relation to the time taken for equilibration of drug between the

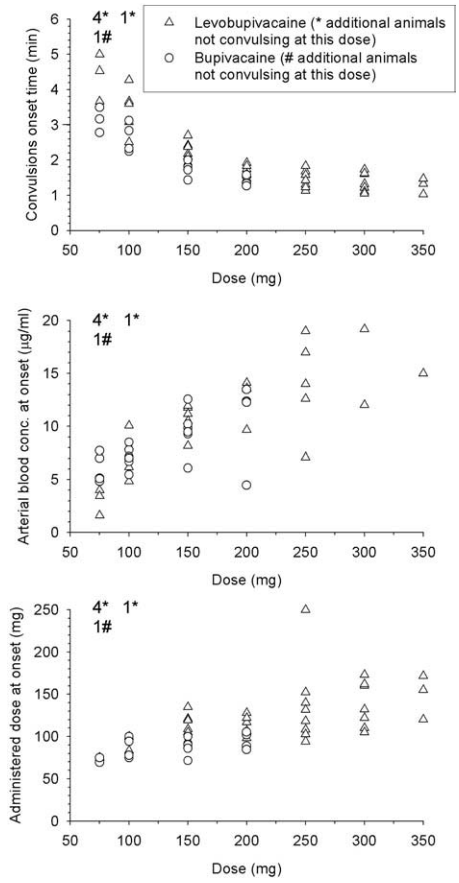

Fig 7. Dose and arterial blood concentration data for the onset of convulsions from intravenous infusion over 3 minutes of bupivacaine or levobupivacaine in a cohort of adult female sheep. Upper panel: time of onset of convulsions plotted against administered dose, middle panel: arterial blood drug concentration at time of onset of convulsions, and lower panel: dose infused to the time of onset of convulsions.

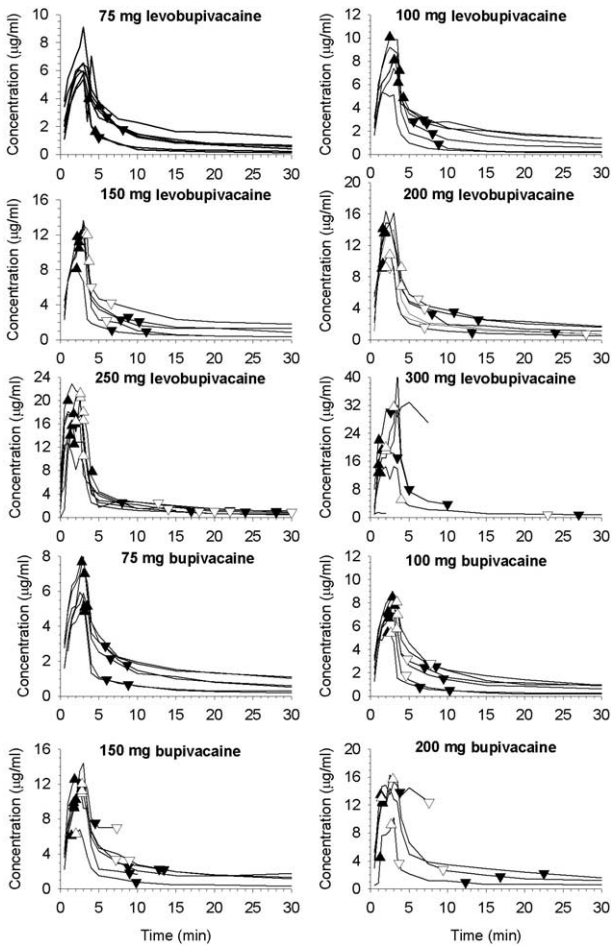

Fig 8. Levobupivacaine and bupivacaine arterial blood drug concentrations resulting from intravenous infusion of the nominated doses over 3 minutes on separate occasions in the same conscious sheep. The points of onset (filled upward triangle) and offset of convulsions (filled downward triangle) and of onset (empty upward triangles) and offset (empty downward triangles) of cardiac dysrhythmias are shown.

(rapidly changing) arterial concentration and tissues. At the lowest dose in particular, the onset occurred later with levobupivacaine after cessation of the 3-minute infusion and when concentrations were decreasing. Moreover, as shown in Figure 8, arterial drug concentrations at the offset of convulsions were always much less than those at the onset, another effect of the experimental conditions. Only a fairly broad range for blood drug concentrations "toxic" to CNS can be described for each local anesthetic as it depends, at least partly, on the conditions under which toxicity occurs.

In contrast, drug concentration in the sagittal sinus at the onset of convulsions does not change markedly with dose rate. As shown in Figure 9, drug concentrations in sagittal sinus blood at the onset and offset of convulsions are similar for each dose and vary far less between doses than do arterial drug concentrations. This indicates that the average "brain" levobupivacaine concentration is reasonably similar at the onset and offset of convulsions (Fig 9). According to venous equilibration theory, the regional venous drug concentration is presumed to better represent the pharmacologically relevant concentration. Thus, the apparent increase in "convulsant drug concentration" for arterial blood with increasing dose appears to be caused by the rate of equilibration across the blood-brain "barrier" being slower than the distribution rate in the rest of the body.

Figure 8 also shows the arterial drug concentrations and the points of onset and offset of periods in which cardiac dysrhythmias occurred. Arterial drug concentrations at the onset of cardiac dysrhythmias differed and were more variable as a function of dose than the corresponding coronary sinus concentrations. This is analogous to the correlation of CNS effects with drug concentrations in sagittal sinus blood. We believe

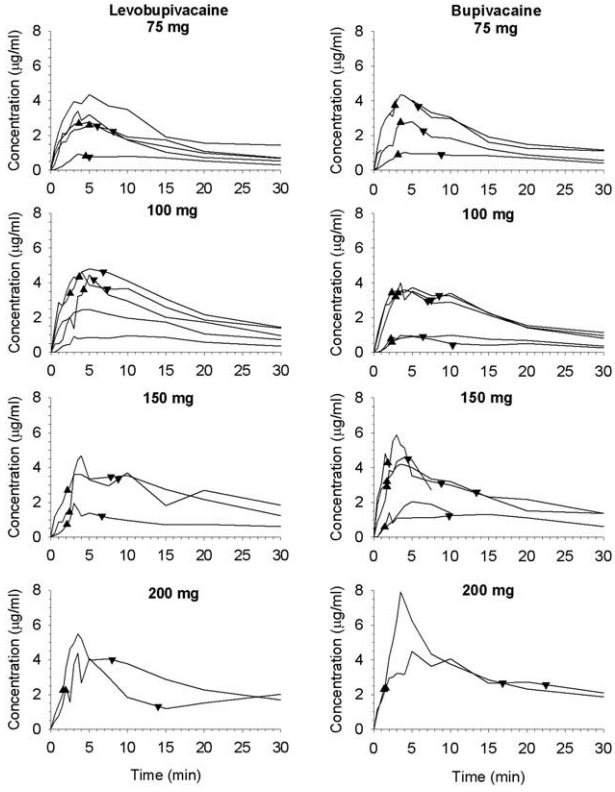

Fig 9. Levobupivacaine and bupivacaine sagittal sinus blood drug concentrations resulting from intravenous infusion of the nominated doses over 3 minutes in the same conscious sheep. The points of onset (filled upward triangle) and cessation of convulsions (filled downward triangle) are shown.

that a similar chaotic paradigm also prevails for CVS effects, leading to the initiation of malignant dysrhythmias. Dose-dependent depression of myocardial contractility occurs in proportion to local anesthetic potency and blood drug concentration until doses are large enough to stimulate the CNS, whereupon the depression is abruptly reversed with the onset of convulsions.[13,14,31,32] Thus, the matter of myocardial intoxication needs to be studied in different settings including conscious and anesthetized subjects, whole-body (intravenous) and site-directed (close arterial) dosing, and intact and isolated tissues to permit a full analysis to be made. This would enable closer examination of the connection between direct (local cardiac) and indirect (mediated by CNS) drug effects. Overall, the role of the CNS by way of sympathetic nervous system stimulation seems paramount; the onset of seizures causes reversal of myocardial depression but predisposes to malignant dysrhythmias. Not surprisingly then, a marked difference in CVS outcome appears to depend on cardiac resting state and state of consciousness.[19,20,32,35,36,39-42]

Speed of Local Anesthetic Injection and Uptake Into Lungs

Slowing the speed of injection and/or dose fractionation is now routine clinical practice: it allows time for reduction of arterial blood drug concentrations delivered to vital organs by concurrent processes of distribution and elimination elsewhere but by how much? Similarly, it has long been known that the lungs exert a "protective" effect against local anesthetic intoxication by attenuating arterial blood drug concentrations,[32] but the magnitude, time course, and dose dependency of this effect has been difficult to evaluate.

It is intuitive that dose fractionation will reduce maximum arterial blood drug concentration (C_{max}), but there does not yet appear to be experimental demonstration of the magnitude of the outcome. We have found experimentally that prolonging from 1 to 3 minutes the intravenous infusion in sheep of 37.5 mg levobupivacaine (as an example) reduces its arterial C_{max} by ~40%, with corresponding reductions in other relevant regions such as the heart as shown by the coronary sinus levobupivacaine concentrations (Fig 10). Computer simula-

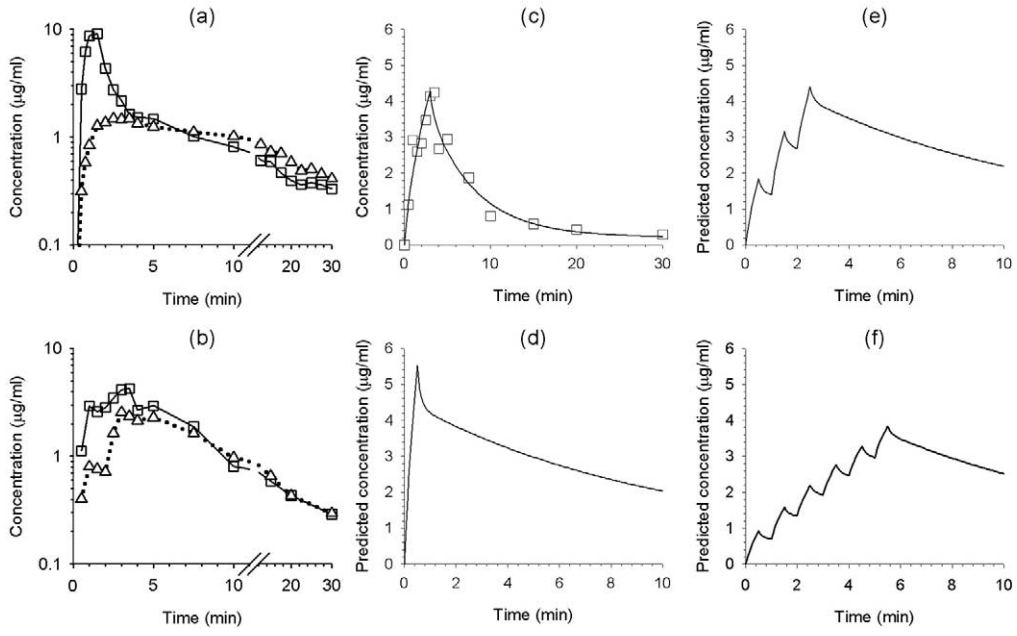

Fig 10. Effect of prolonging the duration of administration of 37.5 mg levobupivacaine administered intravenously. (A) Measured arterial (open squares) and coronary sinus (open triangles) blood levobupivacaine concentrations in a sheep with the dose infused over 1 minute. (B) Measured arterial (open squares) and sagittal sinus (open triangles) blood levobupivacaine concentrations in the same sheep as in A with the dose infused over 3 minutes. (C) Polyexponential curve fitted for pharmacokinetic simulation purposes to the measured arterial blood levobupivacaine concentrations of the subject after 3 minutes of infusion. (D) Simulated arterial blood levobupivacaine concentrations for the dose infused over 30 seconds (no fractionation). (E) Simulated arterial blood levobupivacaine concentrations for the dose infused by fractionation as 3 equal portions, each over 30 seconds, 1 minute apart. (F) Simulated arterial blood levobupivacaine concentrations for the dose infused by fractionation as 6 equal portions, each over 30 seconds, 1 minute apart.

tions of the same dose given as a bolus (30 seconds) or by spaced fractionation into 3 or 6 equal portions shows that the arterial blood C_{max} is attenuated (Fig 10), but this may not be a sufficient reduction in arterial drug concentration to avoid intoxication. The most important feature of dose fractionation is that it gives the anesthesiologist an early opportunity to cease administering the drug if a side effect occurs. The latter aspect is pertinent, given the conflicting views on the suitability of a discrete test dose to prevent intravenous administration of the main dose, particularly for ropivacaine and levobupivacaine, which are less likely than bupivacaine to provoke early CNS symptoms or signs.[43,44] Clearly, in this context, and especially in the CNS-obtunded patient, there is a case to be made for using the response from an epinephrine-containing solution for the entire dose.

Because the lungs are a large organ with complex pathways of transit and tissue uptake, they attenuate arterial blood drug concentrations and, to some extent, consequent risk of local anesthetic intoxication, whether from accidental intravenous administration or systemic absorption after perineural administration,[37,45-47] but by how much and for how long? Some examples of arterial and pulmonary arterial drug concentrations over a large range of levobupivacaine doses in the same sheep are shown in Figure 11. These show that changes in pulmonary arterial drug concentrations precede those in arterial blood during infusion over a large dose range, as expected, and that the gradient reverses soon after cessation of infusion (i.e., net influx is soon followed by net efflux). The lungs attenuate the arterial drug concentrations by ~20%, and the fraction of dose apparently lost into the lungs is essentially independent of dose; however, it is soon regained in an exponentially decreasing manner, and this is a point often not brought out without observation over an extended period of time. Interestingly, we have also found that there are no significant acute differences between bupivacaine enantiomers in sheep or humans in their rate of regional uptake despite a slightly greater tissue binding of the R-bupivacaine enantiomer than S-bupivacaine enantiomer.[46]

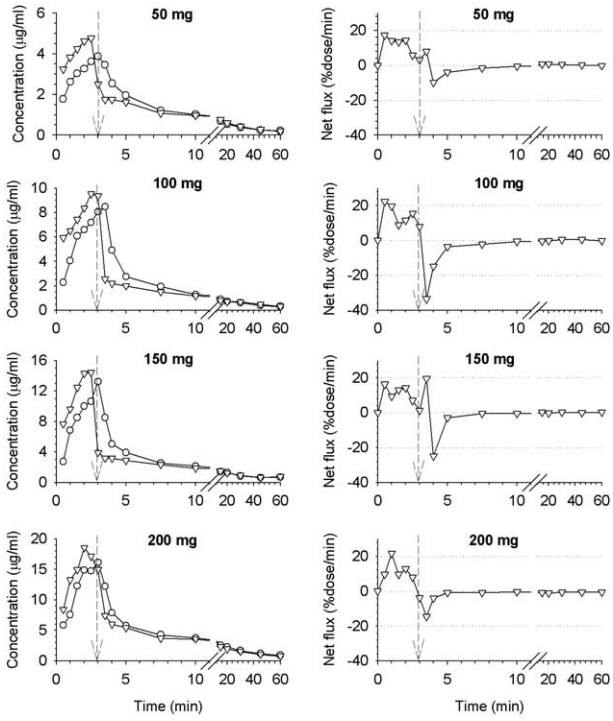

Fig 11. Blood concentration-dose relationships for levobupivacaine after intravenous infusions made over 3 minutes on separate occasions in the same conscious sheep. Left panels: pulmonary arterial (open triangles) and arterial (open circles) blood levobupivacaine concentrations. Right panels: net flux (calculated as % dose/min; net influx is positive, net efflux is negative) of levobupivacaine across the lungs calculated from the product of cardiac output and the difference between pulmonary arterial and arterial drug concentrations. The time of cessation of drug infusion is shown by an arrow.

Protein Binding and the Interpretation of "Blood Drug Concentrations"

A further obstacle in the interpretation of the relationship between circulating drug concentration and drug effects arises from plasma protein binding and blood cell uptake of the drug. This can be even more complex if the drug is administered as a racemate, such as bupivacaine, where the enantiomers can have different protein binding affinities as well as pharmacodynamics. Overall, plasma protein binding opposes blood cell uptake so that blood and plasma drug concentrations will diverge with increased plasma binding.[48] Therefore, the question arises as to whether unbound (or free) drug concentrations (in plasma water) need to be measured for correlation with pharmacological effects or whether total blood or total plasma concentrations give essentially the same information, regardless of species.[11,12,40,49-52] The former are assumed to be equivalent to tissue interstitial fluid and thus in equilibrium with receptor concentrations; unbound concentrations are therefore intuitively attractive, but whole plasma or whole blood concentrations are normally measured because of the lack of a suitable technique for measuring free drug concentrations in most laboratories. In all of the foregoing, it is emphasized that it is the plasma unbound drug *concentration*, not *percentage*, that is the critical issue in driving the pharmacodynamics; there

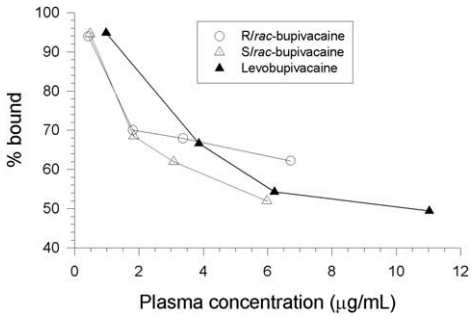

Fig 12. An example of concentration dependence of local anesthetic plasma protein binding showing the individual enantiomers of bupivacaine (from racemate) and levobupivacaine in an individual sheep, showing the unbound fraction is several-fold greater at concentrations associated with central nervous system and cardiovascular system toxicity.

is sometimes confusion on this point and its consequences.[48]

Measurement of unbound drug concentration in plasma now features in many research articles concerned with local anesthetic intoxication in humans and experimental animals. As the current knowledge has accrued over the past few decades, the reporting of plasma-unbound local anesthetic concentrations has been useful to better characterize the pharmacokinetic differences between the drugs (e.g., in transplacental distribution). However, in most cases, the resultant data are a set of numbers scaled differently according to unbound fraction or blood cell uptake that remain closely related to the total plasma drug concentrations of the particular drug, but in a nonlinear manner, and are subject to cumulative methodological errors. With some reservations depending on the use to which the data are being put, it would seem that the total plasma (or blood, as appropriate) drug concentration is a reasonable compromise for most applications.

The most significant feature about the binding of local anesthetics is that its extent is markedly concentration dependent in all species (Fig 12). Thus, the unbound fraction at "toxic" blood drug concentrations can be several times greater than at "nontoxic" concentrations, although it is the latter concentrations at which most drug binding data are generated experimentally. Various plasma proteins have different affinities and capacities to bind different local anesthetics,[53] and the extent of binding is altered by changes of their concentrations, as well as by acid-base changes that affect both the ionization of the drug and conformation of the protein(s). As a further complication, there is some evidence for species-related quantitative differences in binding, which may relate to differential binding to various proteins and their abundance in different species.[52] All such measures are determined generally ex vivo, and they may not reflect well the situation in vivo during a toxic event, when physiology may be altered (e.g., in the immediate postoperative period[51] when concentration of the principal binding protein of local anesthetic [α_1 acid-glycoprotein] changes as a response to stress, and when acid-base and/or fluid balances may be altered).[54]

Sometimes the value of the average blood:plasma drug concentration ratio (B/P, sometimes abbreviated λ) is used to provide a factor for conversion between blood and plasma drug concentrations when one is measured and the other is required for use in a pharmacokinetic calculation.[37] A measured plasma bupivacaine concentration of 3.0 μg/mL in a subject with a B/P ratio of 0.67 would be equivalent to a blood concentration of 2.0 μg/mL because the blood cells occupy a disproportionate volume compared to their drug concentration. It therefore follows that a value of total body clearance as 0.6 L/min determined from measured plasma drug concentrations would be equivalent to a value of 0.9 L/min if calculated from blood samples. A potential hazard of this approach is that B/P itself can be concentration dependent and could depend, theoretically, on other variables such as acid-base balance in the samples assayed, which can alter the ratio of unionized to ionized drug concentrations and protein binding.[54] Nevertheless, it gives a reasonable approximation, as long as concentrations are neither very high nor very low. B/P is also useful as a surrogate for the average drug plasma binding on the premise that greater binding to plasma protein decreases its value.

Animal Research to Human Problems

Some Implications

Prospective studies in humans are mainly used to study pharmacokinetics of local anesthetics after perineural administration, and there are numerous examples of such studies. Intravascular administration to humans for research purposes is permissible only with relatively small doses, with the intention of producing only mild intoxication, and there are a few examples of this type of study. The main purpose of animal research in this context, therefore, is to understand how other mammalian species respond to local anesthetic agents and to use the relevant findings in human medicine.

It has been proposed that maximum recommended doses of local anesthetics should be specified for each administration site rather than for each agent.[1] This is a sensible proposal. Nevertheless, it does not, and

probably cannot, take account of the possibility of accidental intravascular injection and consequent acute local anesthetic intoxication, which remains an ever present risk of neural blockade. Although some clinical reports of acute intoxication include measured blood drug concentrations to verify the probable cause, the amount of pharmacokinetic and pharmacodynamic data available in such circumstances, understandably, is typically sparse. Therefore, to provide greater detail on these matters, this article has focused on some findings from the authors' research into simulated accidental intoxication in conscious sheep and from other models used for the same purpose.

The potency of local anesthetics for causing acute intoxication essentially parallels that for producing neural blockade, with the exceptions of the newer enantiopure agents, ropivacaine and levobupivacaine, which have anesthetic potency similar to bupivacaine but are less toxic. However, the use of these agents does not diminish the risk of their intravascular administration. By-and-large, the gains in safety with these agents are due to more favorable pharmacodynamic profiles than from improved pharmacokinetic characteristics. The risk to the individual from accidental intravascular administration is thus largely proportional to the dose of local anesthetic administered. Safety is promoted by use of the least toxic drug consistent with achieving the desired outcomes of density and duration of nerve block. It is also promoted by safer administration techniques, especially slowing the speed of a local anesthetic injection and/or dose fractionation. Although reduction in blood drug concentrations by these means is undoubtedly beneficial, the main gain in safety may be because of the ability to cease drug administration with less of the dose having been given at the onset of toxic symptoms or signs.

Formal pharmacokinetic-pharmacodynamic investigation of local anesthetic acute intoxication is virtually impossible in humans. Studies in large animals that can be applied to humans can be of immense help in understanding the underlying principles and making comparisons between drugs and between recipients of drugs, especially when pathophysiological perturbations are present. Traditional models of drug effect based on receptor occupancy and monotonic relationships between blood drug concentration and effect do not apply well to local anesthetics, even under controlled laboratory conditions, especially in conscious subjects. Despite this, whenever possible, pharmacokinetic investigation of human intoxication is valuable, but measured blood local anesthetic concentrations in clinical cases of suspected intoxication should be interpreted with some caution and post mortem samples present special hazards, particularly if used to deduce a dosage error.

The other major role of studies in animals is to investigate treatments for intoxication. In the quest to deal with acute local anesthetic intoxication, various strategies have been/are being investigated, some involving pretreatment with pharmacological agents, resuscitation strategies, and even "silver bullet"/selective treatments. This topic is beyond the scope of this article, in which we have outlined more of the relationships between drug structure, dose, pharmacokinetics, and pharmacodynamics that assist in making recommendations about the maximum doses of local anesthetics.

References

1. Rosenberg PH, Veering BT, Urmey WF. Maximum recommended doses of local anesthetics: A multifactorial concept. *Reg Anesth Pain Med* 2004;29:564-575.
2. Mulroy MF. Systemic toxicity and cardiotoxicity from local anesthetics: Incidence and preventive measures. *Reg Anesth Pain Med* 2002;27:556-561.
3. Faccenda KA, Finucane BT. Complications of regional anaesthesia. Incidence and prevention. *Drug Safety* 2001;24:413-442.
4. Weinberg GL. Current concepts in resuscitation of patients with local anesthetic cardiac toxicity. *Reg Anesth Pain Med* 2002;27:568-575.
5. Ruetsch YA, Boni T, Borgeat A. From cocaine to ropivacaine: The history of local anesthetic drugs. *Curr Top Med Chem* 2001;1:175-182.
6. Irita K, Kawashima Y, Morita K, et al. [Critical incidents during regional anesthesia in Japanese Society of Anesthesiologists-Certified Training Hospitals: An analysis of responses to the annual survey conducted between 1999 and 2002 by the Japanese Society of Anesthesiologists]. *Masui* 2005;54:440-449.
7. Buckenmaier CC, Bleckner LL. Anaesthetic agents for advanced regional anaesthesia: A North American perspective. *Drugs* 2005;65:745-759.
8. Mather LE, Long GJ, Thomas J. The intravenous toxicity and clearance of bupivacaine in man. *Clin Pharmacol Ther* 1971;12:935-943.
9. Mather LE, Tucker GT, Murphy TM, Stanton-Hicks MD, Bonica JJ. Cardiovascular and subjective central nervous system effects of long-acting local anaesthetics in man. *Anaesth Intensive Care* 1979;7:215-221.
10. Scott DB, Lee A, Fagan D, Bowler GM, Bloomfield P, Lundh R. Acute toxicity of ropivacaine compared with that of bupivacaine. *Anesth Analg* 1989;69:563-569.
11. Knudsen K, Beckman Suurkula M, Blomberg S, Sjovall J, Edvardsson N. Central nervous and cardiovascular effects of i.v. infusions of ropivacaine, bupivacaine and placebo in volunteers. *Br J Anaesth* 1997;78:507-514.
12. Stewart J, Kellett N, Castro D. The central nervous system and cardiovascular effects of levobupivacaine and ropivacaine in healthy volunteers. *Anesth Analg* 2003;97:412-416.

13. Groban L. Central nervous system and cardiac effects from long-acting amide local anesthetic toxicity in the intact animal model. *Reg Anesth Pain Med* 2003; 28:3-11.
14. Huang YF, Pryor ME, Mather LE, Veering BT. Cardiovascular and central nervous system effects of intravenous levobupivacaine and bupivacaine in sheep. *Anesth Analg* 1998;86:797-804.
15. Chang DH, Ladd LA, Wilson KA, Gelgor L, Mather LE. Tolerability of large-dose intravenous levobupivacaine in sheep. *Anesth Analg* 2000;91:671-679.
16. Chang DH, Ladd LA, Copeland S, Iglesias MA, Plummer JL, Mather LE. Direct cardiac effects of intracoronary bupivacaine, levobupivacaine and ropivacaine in the sheep. *Br J Pharmacol* 2001;132:649-658.
17. Ladd LA, Chang DH, Wilson KA, Copeland SE, Plummer JL, Mather LE. Effects of CNS site-directed carotid arterial infusions of bupivacaine, levobupivacaine, and ropivacaine in sheep. *Anesthesiology* 2002; 97:418-428.
18. Ladd LA, Mather LE. Central Effects Index—A semiquantitative method for the assessment of CNS toxicity of local anaesthetic agents in sheep. *J Pharmacol Toxicol Methods* 2000;44:467-476.
19. Nath S, Häggmark S, Johansson G, Reiz S. Differential depressant and electrophysiologic cardiotoxicity of local anesthetics: An experimental study with special reference to lidocaine and bupivacaine. *Anesth Analg* 1986;65:1263-1270.
20. Morrison SG, Dominguez JJ, Frascarolo P, Reiz S. A comparison of the electrocardiographic cardiotoxic effects of racemic bupivacaine, levobupivacaine, and ropivacaine in anesthetized swine. *Anesth Analg* 2000;90:1308-1314.
21. Fujita Y, Kimura K, Mihira M, Yasukawa T. Decreased coronary blood flow is not responsible for myocardial dysfunction during bupivacaine-induced cardiotoxicity. *Acta Anaesth Scand* 1996;40:216-221.
22. Heavner JE. Cardiac toxicity of local anesthetics in the intact isolated heart model: A review. *Reg Anesth Pain Med* 2002;27:545-555.
23. Pickering AE, Waki H, Headley PM, Paton JF. Investigation of systemic bupivacaine toxicity using the in situ perfused working heart-brainstem preparation of the rat. *Anesthesiology* 2002;97:1550-1556.
24. Heavner JE. Cardiac dysrhythmias induced by infusion of local anesthetics into the lateral cerebral ventricle of cats. *Anesth Analg* 1986;65:133-138.
25. Denson DD, Behbehani MM, Gregg RV. Enantiomer-specific effects of an intravenously administered arrhythmogenic dose of bupivacaine on neurons of the nucleus tractus solitarius and the cardiovascular system in the anesthetized rat. *Reg Anesth* 1992;17:311-316.
26. Åberg G. Toxicological and local anaesthetic effects of optically active isomers of two local anaesthetic compounds. *Acta Pharmacol Toxicol* 1972;31:273-286.
27. Clare JJ, Tate SN, Nobbs M, Romanos MA. Voltage-gated sodium channels as therapeutic targets. *Drug Discov Today* 2000;5:506-520.
28. Mather LE. Studies in the absorption, distribution, metabolism and excretion of drugs by adult and neonatal humans [PhD Thesis]. Sydney, Australia: The University of Sydney, 1972.
29. Åkerman B, Hellberg IB, Trossvik C. Primary evaluation of the local anaesthetic properties of the amino amide agent ropivacaine (LEA 103). *Acta Anaesth Scand* 1988;32:571-578.
30. Vladimirov M, Nau C, Mok WM, Strichartz G. Potency of bupivacaine stereoisomers tested in vitro and in vivo: Biochemical, electrophysiological, and neurobehavioral studies. *Anesthesiology* 2000;93:744-755.
31. Nancarrow C, Rutten AJ, Runciman WB, Mather LE, Carapetis RJ, McLean CF, Hipkens SF. Myocardial and cerebral drug concentrations and the mechanisms of death after fatal intravenous doses of lidocaine, bupivacaine, and ropivacaine in the sheep. *Anesth Analg* 1989;69:276-283.
32. Groban L, Deal DD, Vernon JC, James RL, Butterworth J. Does local anesthetic stereoselectivity or structure predict myocardial depression in anesthetized canines? *Reg Anesth Pain Med* 2002;27:460-468.
33. Upton RN, Mather LE, Runciman WB, Nancarrow C. The use of mass balance principles to determine regional drug distribution and elimination. *J Pharmacokinet Biopharm* 16:13-29, 1988.
34. Runciman WB, Myburgh J, Upton RN, Mather LE. Effects of anaesthesia on drug disposition. In: Feldman SA, Scurr CF, Paton W, eds. *Mechanisms of Action of Drugs in Anaesthetic Practice*. 2nd ed. London: Edward Arnold;1993:83-128.
35. Ladd LA, Copeland SE, Mather LE. Neurally-mediated cardiotoxicity of local anesthetics: Direct effect of seizures or of local anesthetics? *Anesthesiology* 2003;98:1296-1297.
36. Mather LE, Duke CC, Ladd LA, Copeland SE, Gallagher G, Chang DH. Direct cardiac effects of coronary site-directed thiopental and its enantiomers: A comparison to propofol in conscious sheep. *Anesthesiology* 2004;101:354-364.
37. Tucker GT, Mather LE. Pharmacology of local anaesthetic agents. Pharmacokinetics of local anaesthetic agents. *Br J Anaesth* 1975;47(suppl):213-224.
38. Feldman HS, Hartvig P, Wiklund L, Doucette AM, Antoni G, Gee A, Ulin J, Langstrom B. Regional distribution of ^{11}C-labeled lidocaine, bupivacaine, and ropivacaine in the heart, lungs, and skeletal muscle of pigs studied with positron emission tomography. *Biopharm Drug Dispos* 1997;18:151-164.
39. Huang YF, Upton RN, Runciman WB. IV bolus administration of subconvulsive doses of lignocaine to conscious sheep: Relationships between myocardial pharmacokinetics and pharmacodynamics. *Br J Anaesth* 1993;70:556-561.
40. Santos AC, DeArmas PI. Systemic toxicity of levobupivacaine, bupivacaine, and ropivacaine during continuous intravenous infusion to nonpregnant and pregnant ewes. *Anesthesiology* 2001;95:1256-1264.
41. Lefrant JY, de La Coussaye JE, Ripart J, et al. The comparative electrophysiologic and hemodynamic

effects of a large dose of ropivacaine and bupivacaine in anesthetized and ventilated piglets. *Anesth Analg* 2001;93:1598-1605.
42. Aya AG, de la Coussaye JE, Robert E, et al. Comparison of the effects of racemic bupivacaine, levobupivacaine, and ropivacaine on ventricular conduction, refractoriness, and wavelength: An epicardial mapping study. *Anesthesiology* 2002;96:641-650.
43. McCartney CJ, Murphy DB, Iagounova A, Chan VW. Intravenous ropivacaine bolus is a reliable marker of intravascular injection in premedicated healthy volunteers. *Can J Anaesth* 2003;50:795-800.
44. Owen MD, Gautier P, Hood DD. Can ropivacaine and levobupivacaine be used as test doses during regional anesthesia? *Anesthesiology* 2004;100:922-925.
45. Kietzmann D, Foth H, Geng WP, Rathgeber J, Gundert-Remy U, Kettler D. Transpulmonary disposition of prilocaine, mepivacaine, and bupivacaine in humans in the course of epidural anaesthesia. *Acta Anaesth Scand* 1995;39:885-890.
46. Sharrock NE, Mather LE, Go G, Sculco TP. Arterial and pulmonary arterial concentrations of the enantiomers of bupivacaine after epidural injection in elderly patients. *Anesth Analg* 1998;86:812-817.
47. Ohmura S, Sugano A, Kawada M, Yamamoto K. Pulmonary uptake of ropivacaine and levobupivacaine in rabbits. *Anesth Analg* 2003;97:893-897.
48. Tucker GT. Is plasma binding of local anesthetics important? *Acta Anaesth Belg* 1988;39:147-150.
49. Veering BT, Burm AGL, Feyen HM, Olieman W, Souverijn JHM, Van Kleef JW. Pharmacokinetics of bupivacaine during postoperative epidural infusion: Enantioselectivity and role of protein binding. *Anesthesiology* 2002;96:1062-1069.
50. Groban L, Deal DD, Vernon JC, James RL, Butterworth J. Cardiac resuscitation after incremental overdosage with lidocaine, bupivacaine, levobupivacaine, and ropivacaine in anesthetized dogs. *Anesth Analg* 2001;92:37-43.
51. Rutten AJ, Mather LE, Plummer JL, Henning EC. Postoperative course of plasma protein binding of lignocaine, ropivacaine and bupivacaine in sheep. *J Pharm Pharmacol* 1992;44:355-358.
52. Coyle DE, Denson DD, Thompson GA, Myers JA, Arthur GR, Bridenbaugh PO. The influence of lactic acid on the serum protein binding of bupivacaine: Species differences. *Anesthesiology* 1984;61:127-133.
53. Mather LE, Thomas J. Bupivacaine binding to plasma protein fractions. *J Pharm Pharmacol* 1978;30:653-654.
54. Mather LE, Ladd LA, Chang DH-T, Copeland SE. The effects of imposed acid-base derangement on the cardio-activity and pharmacokinetics of bupivacaine and thiopental. *Anesthesiology* 2004;100:1457-1468.