

◆ Translational Vignette

Scavenging Nanoparticles: An Emerging Treatment for Local Anesthetic Toxicity

Elizabeth M. Renehan, M.D., M.Sc., F.R.C.P.C., F. Kayser Enneking, M.D., Manoj Varshney, Ph.D., Richard Partch, Ph.D., Donn M. Dennis, M.D., F.A.H.A., and Timothy E. Morey, M.D.

A dreaded complication of local anesthetic use in regional anesthesia is systemic toxicity from unintentional intravascular local anesthetic injection. Although systemic toxic reactions are not common after peripheral nerve block, 7.5 to 20 events per 10, 000 in adults,¹ they can be life-threatening and resistant to treatment.²⁻⁶ Recent data from an American Society of Anesthesiology Closed Claims Project demonstrate that unintentional intravenous local anesthetic injection was the second largest category of block-related regional anesthesia claims that resulted in death or brain damage.⁷

The manifestations of local anesthetic toxicity range from local neurotoxic and myotoxic reactions to cardiovascular collapse and coma. Central nervous system toxicity presents as a spectrum that includes shivering, muscle twitching, tonic-clonic seizures, hypoventilation, and respiratory arrest.⁸ Cardiovascular toxicity primarily manifests as arrhythmias and myocardial depression.⁹⁻¹⁴ Local anesthetic overdose can cause a variety of arrhythmias, including atrial and ventricular conduction delays, complete heart block, asystole, ventricular ectopy, ventricular tachycardia, torsades de pointes, and ventricular fibrillation.^{8,15}

The molecular mechanisms whereby local anesthetics exert their toxic effects have not been fully established and are likely complex in nature. Ex-

perimental studies have revealed that local anesthetics alter a wide variety of cellular signal transduction and metabolic processes. These drugs modify ion flux at sodium, potassium, and calcium channels.¹⁶⁻¹⁹ They also alter ligand binding to β_2 -adrenergic receptors and, as a result, inhibit cAMP second-messenger signaling.^{20,21} Other potential sites of action include ion channel-linked G-protein pathways, mitochondrial ATP production, and endothelial nitric oxide release.²²⁻²⁴ Which, if any, of these molecular mechanisms (or a combination thereof) are responsible for the profound cardiovascular depression associated with anesthetic overdose remains unknown. In fact, the ability of local anesthetics to interfere with cellular function and homeostasis concurrently at multiple levels may be the factor that makes overdose with these drugs potentially severe and refractory.

Traditionally, treatment of patients who experience severe local anesthetic-induced systemic toxicity relies on supportive measures. Rapid provision of adequate oxygenation, ventilation, seizure control, and cardiovascular support according to ACLS guidelines are fundamental to restoring homeostasis. Less clear are the roles of specific antiarrhythmic agents, vasopressors, and positive inotropes in the resuscitation of local anesthetic-induced cardiotoxicity. The results of animal studies of epinephrine, norepinephrine, amrinone, amiodarone, milrinone, and vasopressin in the treatment of bupivacaine or ropivacaine-induced cardiotoxicity have not been in complete accord.¹⁵ On the basis of the balance of data in this setting, several authors have advocated the use of vasopressin over epinephrine for support of cardiac output, amiodarone over lidocaine for treatment of severe arrhythmias, and the avoidance of calcium channel blockers and phenytoin.^{15,25} A role for emergent cardiopulmonary bypass in the treatment of local anesthetic-induced refractory cardiac arrest has also been proposed.²⁶

In addition to these conventional therapies, three

From the Departments of Anesthesiology, and Orthopedics and Rehabilitation, University of Florida, Gainesville, FL; Particle Engineering Research Center, University of Florida, Gainesville, FL, USA; Department of Chemistry, Clarkson University, Potsdam, NY, USA; and Department of Pharmacology and Experimental Therapeutics, University of Florida, Gainesville, FL.

Accepted for publication April 5, 2005.

Reprint requests: Timothy E. Morey, M.D., Department of Anesthesiology, PO Box 100254, Gainesville, FL 32610-0254. E-mail: morey@ufl.edu

© 2005 by the American Society of Regional Anesthesia and Pain Medicine.

1098-7339/05/3004-0001\$30.00/0

doi:10.1016/j.rapm.2005.04.004

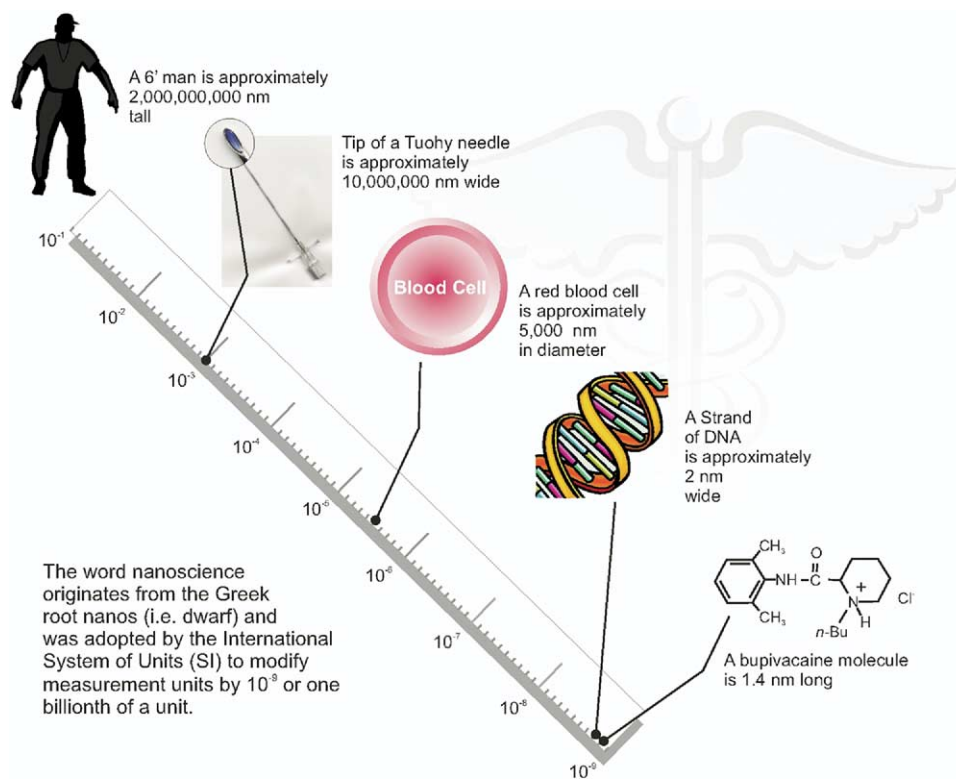


Fig 1. Nanoscale size comparisons.

novel treatments for severe bupivacaine cardiotoxicity in animals have been published: insulin/glucose infusion, propofol infusion, and lipid infusion.²⁷⁻³⁰ Of these, the work on lipid-based resuscitation is directly relevant to the use of drug-scavenging nanoparticles in the treatment of bupivacaine cardiotoxicity. In two studies, Weinberg et al.²⁹ reported a beneficial effect of lipid infusion on bupivacaine-induced cardiotoxicity. In rats, pretreatment with a lipid infusion increased the bupivacaine dose required to induce asystole in rats by 48% over control animals. A subsequent study in dogs showed that a lipid infusion during resuscitation from bupivacaine-induced circulatory collapse increased survival from no animals in the control group to all animals in the lipid-treated group.³⁰ Although these results are promising, no published reports of lipid use for treatment of bupivacaine toxicity in humans exist.

The authors of the lipid-based studies speculated that four mechanisms may play a role in the success of resuscitation. In their primary hypothesis, the lipid infusion may create plasma lipid droplets capable of segregating uncharged bupivacaine molecules from plasma, which makes them unavailable for interaction at their target sites. The authors supported this theory by showing that bupivacaine molecules preferentially segregated from plasma to

their lipid infusion in a 1:12 ratio.²⁹ In two of the other proposed mechanisms, the lipid acts within tissue. Here, lipid or its component fatty acids either interact in a clinically significant way with tissue bupivacaine molecules or directly overcome bupivacaine's inhibitory effect on cellular metabolism by supplying substrate for cellular energy production.^{30,31} Finally, the lipid infusion may act on nitric oxide pathways and reverse bupivacaine's inhibitory effects.²⁹ Building on this work and assuming that sequestration of bupivacaine is an important aspect of resuscitation in the aforementioned lipid-based studies, some investigators have hypothesized even greater segregation of bupivacaine into lipid may occur with large reductions in particle size to the dimension of the nanometer.

Nanotechnology

The word nanoscience originates from the Greek root *nanos* (i.e., dwarf) and was adopted by the International System of Units (SI) to modify measurement units by 10^{-9} or one billionth of a unit. On a biological scale familiar to clinicians, a person is about 2,000,000,000 nm tall, a red blood cell is approximately 5,000 nm in diameter, a DNA strand is approximately 2 nm wide, and a bupivacaine molecule is 1.4 nm long (Fig 1).³¹ Nanoscience is

the study, development, and use of matter at the nanometer scale (1 to 100 nanometers). Originally foreseen by physicist and Nobel laureate Richard P. Feynman in 1959, nanoscience has vaulted into scientific and popular awareness over the past decade as the technology to create nanoscale objects has evolved. That is, whereas engineers and others have reduced the sizes of objects from micrometer to nanometer size (“top-down”), chemists and others have worked to develop mechanisms for individual molecules to self-assemble into nanoparticles (“bottom-up”). Nanoscience is a fundamentally novel way of using matter because, at nanometer size, matter develops unique, previously unrecognized properties. For example, the interaction of nanometer-sized particles with each other or with their environment is primarily influenced by surface tension and local electromagnetic effects, rather than by gravity or electrostatic forces. Although these unique physical properties and others have not yet been fully characterized, they are beginning to be applied in many economic sectors, such as the textile industry, information technology, the military, health care, and others.

Drug Scavenging Nanoparticles in Bupivacaine Cardiac Toxicity

Given the experimental success of lipid-based resuscitation, Morey et al.³² proposed that reducing the particle size of the bupivacaine sink would significantly increase the aggregate surface area of the particles. As a result, the particles’ efficiency in extracting bupivacaine from blood would be similarly increased.^{32,33} In fact, the resulting oil-in-water emulsion-based nanoparticles that were synthesized had approximate diameters of 15 to 120 nm, roughly one quarter the size of a 400-nm diameter Intralipid (Pharmacia & Upjohn, Kalamazoo, MI) molecule (Fig 2).^{32,34} In addition, the increases in the total number of nanoparticles of 15 to 120 nm more than compensated for the reduction in surface area of single particles by reductions in diameter from 400 nm. Expressing this idea mathematically:

$$(1) \quad SA_{\text{AggPart}} = N_{\text{Part}} \cdot SA_{\text{Part}}$$

$$(2) \quad SA_{\text{Part}} = 4\pi \left(\frac{P_{\text{Dia}}}{2} \right)^2$$

where SA_{AggPart} is the aggregate surface area of the particles, N_{Part} is the number of particles, SA_{Part} is the surface area of a particle, and P_{Dia} is the diameter of a particle. For almost all nanostructured materials, an important property is that the components of the particle are essentially at the surface or

very close to the surface of the individual nanoparticle. Thus, these larger aggregate surface areas could potentially be used to sequester local anesthetic molecules, with subsequent “sinking” into an interior lipid core.

A series of experiments with these emulsion-based nanoparticles investigated whether their reduced size translated into a measurable improvement in bupivacaine extraction. In a simple phosphate-buffered saline solution, the smaller nanoparticles sequestered a greater mass of bupivacaine from the free phase than did Intralipid.³⁵ Removal of bupivacaine was markedly enhanced by addition of fatty acid sodium salts that resulted in greater oil/water interface area, increased columbic interaction between bupivacaine and fatty acids sodium salt, and greater surface activity.³⁴ Extraction of bupivacaine increased approximately 4-fold as the alkyl chain was lengthened from 0 to 18 carbon atoms, although this effect maximized at 14 carbon atoms. In addition, the cblock Pluronic (BASF, Florham Park, NJ) polymer type and concentration also significantly affected bupivacaine extraction, probably by the variance in the total number of hydrophobic moieties and the ratio of hydrophobic: hydrophilic blocks within a specific polymer used. Pluronic polymers are symmetric triblock copolymers of propylene oxide (PO) and ethylene oxide (EO). The polypropylene oxide block is sandwiched between the more hydrophilic polyethylene oxide blocks. The block copolymer is denoted by the $(EO)_x(PO)_y(EO)_x$, where x and y are the number of units of EO and PO, respectively. Furthermore, the fractional occupancy of these particles can be calculated as

$$(3) \quad \text{Volume}(\%) = 3.15 \cdot 10^4 \frac{\text{Drugmoles}_{\text{ME}}}{Y} \cdot Z_d^3$$

where Z_d is the diameter of drug in nm, Y is the concentration of particles, and $\text{Drugmoles}_{\text{ME}}$ is the measured moles of drug in or on the particles. Given a constant amount of oil and surfactant, the fractional occupancy of the particles by bupivacaine increased dramatically from 2.0% to 40.3% with reductions in particle diameter from 430 ± 10 nm (Intralipid) to 29 ± 2 nm (experimental nanoparticles).³⁴ Interestingly, the mass of oil had minimal to no effect on bupivacaine extraction compared with the quantity and exact characteristics of both the fatty acid and cblock polymer surfactants in a buffered phosphate solution. In human whole blood, these nanoparticle emulsions also reduced the free concentration of bupivacaine and did so in a concentration-dependent manner. Subsequent refinements in design showed that increases in the

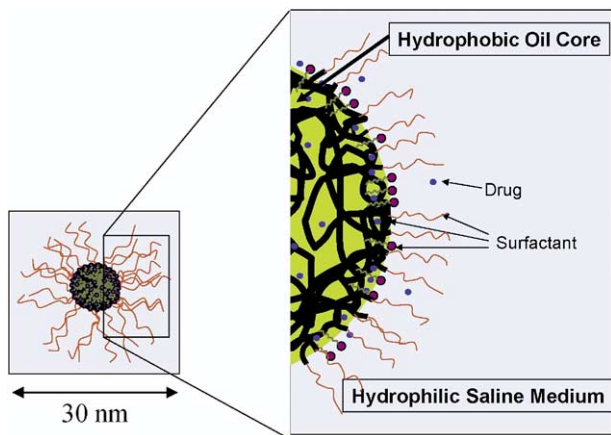


Fig 2. Extraction of bupivacaine by nanoparticles. Shown is an oil-in-water emulsion-based nanoparticle composed of an oil (yellow core) and 2 surfactants in a normal saline bulk medium. The surfactants incorporated into the nanoparticle are a coblock nonionic polymer composed of ethylene oxide (black threads) and propylene oxide (red threads), as well as the ionic surfactant sodium caprylate (polar purple circles with aliphatic gray tails). The blue circles represent drug molecules, such as bupivacaine, and are shown partitioning from the hydrophilic medium into the nanoparticle's hydrophobic core. (Redrawn from Varshney et al., *J Am Chem Soc* 2004;126: 5108-5112. Used by permission.)

mass of ionic surfactant allowed even more efficacious extraction of bupivacaine with concurrent reductions in nanoparticle size.³³

The hypothesis that decreasing the bupivacaine sink particle size leads to more efficient bupivacaine extraction has also been validated in an isolated heart model of bupivacaine cardiac toxicity.³² In this model, the intraventricular conduction time (QRS interval) of isolated guinea pig heart acts as a biosensor of local anesthetic concentration. As the free bupivacaine concentration in the solution bathing the heart increases, so does the QRS interval. This relationship between QRS interval and bupivacaine concentration exists because intraventricular conduction time depends on sodium-channel ion flux, which, in turn, is blocked by local anesthetics in a concentration-dependent manner. When the nanoparticle emulsions were compared with Intralipid in this model, the nanoparticles attenuated bupivacaine toxicity more effectively and more rapidly. That is, whereas the QRS interval prolongation caused by bupivacaine was attenuated approximately 25% by Intralipid, it was completely abolished by nanoparticles after 5 minutes.³⁵ Additional studies to determine the effects of these nanoparticles in living rodent intoxicated with local anesthetic are ongoing. However, use of similar nanoparticles to treat animals poisoned with an-

other sodium-channel antagonist, amitriptyline, have been conducted. In these experiments, nanoparticles attenuated amitriptyline-induced prolongation of the QRS interval to a significantly greater extent than did Intralipid.³⁶ On the basis of the *in vitro* and *in vivo* evidence, nanoparticles do seem to sequester these lipophilic sodium-channel blockers (i.e., bupivacaine and amitriptyline) to a greater extent than does Intralipid and to attenuate the cardiac effects of these drugs.

The therapeutic application of nanoscience as a potential treatment for the cardiotoxic effects of local anesthetics may be useful in the future. A large amount of ongoing research exploring therapeutic and diagnostic applications for this technology is underway in both academic and private settings. We believe the nanotechnology revolution will fundamentally change many aspects of health-care delivery and the practice of medicine.

References

- Mulroy MF. Systemic toxicity and cardiotoxicity from local anesthetics: Incidence and preventive measures. *Reg Anesth Pain Med* 2002;27:556-561.
- Albright GA. Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. *Anesthesiology* 1979;51:285-287.
- Long WB, Rosenblum S, Grady IP. Successful resuscitation of bupivacaine-induced cardiac arrest using cardiopulmonary bypass. *Anesth Analg* 1989;69:403-406.
- Conklin KA, Ziadlou-Rad F. Bupivacaine cardiotoxicity in a pregnant patient with mitral valve prolapse. *Anesthesiology* 1983;58:596.
- Gould DB, Aldrete JA. Bupivacaine cardiotoxicity in a patient with renal failure. *Acta Anaesthesiol Scand* 1983;27:18-21.
- Fortuna A, Fortuna AO. Bupivacaine-induced cardiac arrest. *Anesth Analg* 1990;71:561-562.
- Lee LA, Posner KL, Domino KB, Caplan RA, Cheney FW. Injuries associated with regional anesthesia in the 1980s and 1990s: A closed claims analysis. *Anesthesiology* 2004;101:143-152.
- 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.
- Kotelko DM, Shnider SM, Dailey PA, Brizgys RV, Levinson G, Shapiro WA, Koike M, Rosen MA. Bupivacaine-induced cardiac arrhythmias in sheep. *Anesthesiology* 1984;60:10-18.
- Pitkanen M, Feldman HS, Arthur GR, Covino BG. Chronotropic and inotropic effects of ropivacaine, bupivacaine, and lidocaine in the spontaneously beating and electrically paced isolated, perfused rabbit heart. *Reg Anesth* 1992;17:183-192.

11. Partridge BL. The effects of local anesthetics and epinephrine on rat sciatic nerve blood flow. *Anesthesiology* 1991;75:243-250.
12. de La Coussaye JE, Brugada J, Alessie MA. Electrophysiologic and arrhythmogenic effects of bupivacaine: A study with high-resolution ventricular epicardial mapping in rabbit hearts. *Anesthesiology* 1992;77:132-141.
13. Lacombe P, Blaise G, Loulmet D, Hollmann C. Electrophysiologic effects of bupivacaine in the isolated rabbit heart. *Anesth Analg* 1991;72:62-69.
14. Groban L, Deal DD, Vernon JC, James RL, Butterworth J. Cardiac resuscitation after incremental overdose with lidocaine, bupivacaine, levobupivacaine, and ropivacaine in anesthetized dogs. *Anesth Analg* 2001;92:37-43.
15. Weinberg GL. Current concepts in resuscitation of patients with local anesthetic cardiac toxicity. *Reg Anesth Pain Med* 2002;27:568-575.
16. Valenzuela C, Delpon E, Tamkun MM, Tamargo J, Snyders DJ. Stereoselective block of a human cardiac potassium channel (Kv1.5) by bupivacaine enantiomers. *Biophys J* 1995;69:418-427.
17. Clarkson CW, Hondeghem LM. Mechanism for bupivacaine depression of cardiac conduction: Fast block of sodium channels during the action potential with slow recovery from block during diastole. *Anesthesiology* 1985;62:396-405.
18. Castle NA. Bupivacaine inhibits the transient outward K⁺ current but not the inward rectifier in rat ventricular myocytes. *J Pharmacol Exp Ther* 1990;255:1038-1046.
19. Xiong Z, Strichartz GR. Inhibition by local anesthetics of Ca²⁺ channels in rat anterior pituitary cells. *Eur J Pharmacol* 1998;363:81-90.
20. Butterworth JF, Brownlow RC, Leith JP, Prielipp RC, Cole LR. Bupivacaine inhibits cyclic-3',5'-adenosine monophosphate production: A possible contributing factor to cardiovascular toxicity. *Anesthesiology* 1993;79:88-95.
21. Butterworth J, James RL, Grimes J. Structure-affinity relationships and stereospecificity of several homologous series of local anesthetics for the beta₂-adrenergic receptor. *Anesth Analg* 1997;85:336-342.
22. Zhou W, Arrabit C, Choe S, Slesinger PA. Mechanism underlying bupivacaine inhibition of G protein-gated inwardly rectifying K⁺ channels. *Proc Natl Acad Sci USA* 2001;98:6482-6487.
23. Xiong Z, Bukusoglu C, Strichartz GR. Local anesthetics inhibit the G protein-mediated modulation of K⁺ and Ca⁺⁺ currents in anterior pituitary cells. *Mol Pharmacol* 1999;55:150-158.
24. Johns RA. Local anesthetics inhibit endothelium-dependent vasodilation. *Anesthesiology* 1989;70: 805-811.
25. Groban L, Butterworth J. Lipid reversal of bupivacaine toxicity: Has the silver bullet been identified? *Reg Anesth Pain Med* 2003;28:167-169.
26. Soltész EG, van Pelt F, Byrne JG. Emergent cardiopulmonary bypass for bupivacaine cardiotoxicity. *J Cardiothorac Vasc Anesth* 2003;17:357-358.
27. Cho HS, Lee JJ, Chung IS, Shin BS, Kim JA, Lee KH. Insulin reverses bupivacaine-induced cardiac depression in dogs. *Anesth Analg* 2000;91:1096-1102.
28. Ohmura S, Ohta T, Yamamoto K, Kobayashi T. A comparison of the effects of propofol and sevoflurane on the systemic toxicity of intravenous bupivacaine in rats. *Anesth Analg* 1999;88:155-159.
29. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ. Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. *Anesthesiology* 1998;88:1071-1075.
30. Weinberg G, Ripper R, Feinstein DL, Hoffman W. Lipid emulsion infusion rescues dogs from bupivacaine-induced cardiac toxicity. *Reg Anesth Pain Med* 2003;28:198-202.
31. Courtney KR. Why do some drugs preferentially block open sodium channels? *J Mol Cell Cardiol* 1988;20:461-464.
32. Morey TE, Varshney M, Flint JA, Rajasekaran S, Shah DO, Dennis DM. Treatment of local anesthetic-induced cardiotoxicity using drug scavenging nanoparticles. *Nano Lett* 2004;4:757-759.
33. Varshney M, Morey TE, Shah DO, Flint JA, Moudgil BM, Seubert CN, Dennis DM. Pluronic microemulsions as nanoreservoirs for extraction of bupivacaine from normal saline. *J Am Chem Soc* 2004;126:5108-5112.
34. Varshney M, Morey TE, Shah DO, Flint JA, Moudgil BM, Seubert CN, Dennis DM. Pluronic microemulsions as nanoreservoirs for extraction of bupivacaine from normal saline. *J Am Chem Soc* 2004;126:5108-5112.
35. Morey TE, Varshney M, Flint JA, Rajasekaran S, Shah DO, Dennis DM. Treatment of local anesthetic-induced cardiotoxicity using drug scavenging nanoparticles. *Nano Lett* 2004;4:757-759.
36. Morey TE, Varshney M, Flint JA, Wawrzyniak JM, Dennis DM. Attenuation of the cardiotoxic effects of bupivacaine in anesthetized rat by nanoparticles [Abst.]. *Anesthesiology* 101;A1100:2004.