“Noninvasive” Testing for Malignant Hyperthermia Susceptibility

MALIGNANT hyperthermia (MH) is probably the only medical syndrome that can only be caused by the administration of anesthetic agents. Because it is almost uniformly fatal if not rapidly diagnosed and treated, it would be a significant improvement for anesthesia risk management if we could reliably predict or confirm MH susceptibility.

The mechanism of MH appears to be a defect in skeletal muscle excitation-contraction coupling. In 50% of patients, MH has been associated with one of 20 or more mutations in the type 1 ryanodine receptor (RyR1), the skeletal muscle sarcoplasmic reticulum Ca2+ release channel, and in one family it was associated with a mutation in the α1s-dihydropyridine receptor, the slow voltage gated calcium channel in the sarcolemma. However, in the remaining 50% of patients it has not yet been linked to any genetic locus. This seriously limits our ability to use genetic testing methods. The diagnosis of MH is still a result of a clinical event, and these events are associated with variable symptoms. There have been efforts to grade these events and to make the diagnosis based on a clinical score, but it would be very desirable to have a simple laboratory test that could predict or confirm MH susceptibility with a high level of confidence. Over the years, there have been many diagnostic tests that have been proposed to predict MH susceptibility. However, only the caffeine, caffeine-halothane, and 4 chloro-m-creosol (4-CmC) contracture tests (Caffeine Halothane Contracture Test [CHCT] in North America and In Vitro Contracture Test [IVCT] in Europe) have withstood the test of time. The CHCT is not perfect, but it has had a high degree of correlation with genetic testing in the small, clinically selected population of individuals susceptible to MH. Nevertheless, contracture testing requires a large surgical muscle biopsy and, although readily available in Europe, is performed at a decreasing number of centers in the United States. A less invasive test that could be easily performed at any center and would be at least as reliable as the CHCT would obviously be very desirable. In this issue of Anesthesiology, there are two new tests proposed by Sei et al. and Klingler et al. that are “noninvasive” and appear to correlate well with both the CHCT and genetic testing. If the results of these preliminary studies are confirmed, the new tests may well prove to be a useful adjunct to the diagnosis of MH susceptibility. If not, they still suggest new paths to be explored in the direction of this “holy grail.”

Sei et al. had previously demonstrated the existence of RyR1 in B lymphocytes. Girard et al. demonstrated that B lymphocytes from patients susceptible to MH produced more interleukin 1β in response to caffeine and 4-CmC than those from patients who were not susceptible to MH. Based on these findings and the recent findings of others, Sei et al. hypothesized that Ca2+ homeostasis in B lymphocytes is altered in individuals who are susceptible to MH and proposed that this could be the basis for a noninvasive diagnostic blood test. The current study by Sei et al. shows that Ca2+ release induced by caffeine and 4-CmC in cells from individuals susceptible to MH is greater than that in patients with normal CHCT test results (MH susceptibility negative) and that in normal control subjects who did not undergo CHCT testing. This suggests that Ca2+ regulation may be altered in B lymphocytes, and that this malfunction is related to the mutation of RyR1. In addition, on average, the MH-susceptible cells show a lower threshold for 4-CmC than controls. The finding that the caffeine response can be abolished by treatment with EGTA or Ca2+-free media was unexpected, since caffeine induces its pharmacologic action in the vast majority of cells not by facilitating the entry of Ca2+ from the extracellular space, but by releasing Ca2+ from intracellular stores. This will require further study. However, the lack of a clear-cut point between the responses from cells that are susceptible to MH and those that are not, which is probably related to the heterogeneity of the MH phenotype, limits our ability to discriminate between patients who are and are not susceptible to MH. It will also be necessary for Sei et al. to determine whether this test will have any value for use in patients who do not have a mutation of the RyR1, since B lymphocytes have not been shown to express the other proteins thought to be important for skeletal excitation-contraction coupling or MH. While these factors limit the current utility of this test for diagnosing MH susceptibility, we look forward to

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further work in this area, with the hope that a more usable clinical test might be developed.

In the report by Klingler et al.11 in this issue, the investigators have proposed a clever extension of the IVCT. They propose (but have not shown) that this can be done from the tissue collected from a small needle biopsy of muscle, eliminating the need for a surgical procedure and making the test almost “noninvasive.” They use the premise that proton excretion from muscle, in this case human myotubes, is the result of increased metabolic rate, and the larger the rate of proton excretion for a given stimulus, the greater the metabolic activity. Despite the fact that this is an indirect measurement, 4-CmC is highly specific for stimulating Ca$^{2+}$ release from RyR1,14 which, in turn, will increase the metabolic rate. Thus, any increase in proton release caused by 4-CmC is likely to be the result of Ca$^{2+}$ metabolism. The myotubes that Klingler et al.11 cultured were not from needle biopsies but from large biopsy samples taken for IVCT testing. This did have the advantage of allowing direct correlation of the two techniques but does not validate the proposed needle biopsy technique. From their data, they show a reasonable correlation between the threshold for proton excretion and force production for 4-CmC and a complete separation of MH susceptibility and lack of MH susceptibility using these criteria. If this test can actually be performed on needle biopsy samples, it is a big step forward in decreasing the invasiveness of MH testing, and, since it is performed on muscle, it is likely to be as useful as the IVCT (CHCT) in diagnosing individuals susceptible to MH.

Despite this step forward, it is our opinion that there is one major problem facing all diagnostic tests for MH that use skeletal muscle responses. Despite the fact that we all want to know the results of such a test, we have found, in a show-of-hands poll of more than 800 anesthesiologists after MH lectures, that simply performing the test is sufficient for 99% of the respondents in the United States to consider the patient tested to be susceptible to MH—no matter what the result! Furthermore, the CHCT (IVCT) is not a specific test for MH in and of itself. Increasing myoplasmic free Ca$^{2+}$ concentration in muscle that is not susceptible to MH will reversibly convert its phenotype to MH susceptible,15 and we have recently shown that lowering the myoplasmic free Ca$^{2+}$ concentration in MH-susceptible muscle can reversibly convert its phenotype to MH susceptibility negative (J. R. López, M.D., Ph.D., and P. D. Allen, M.D., Ph.D., unpublished data, June 2002). Several other muscle diseases besides MH have increased caffeine sensitivity due to a high resting Ca$^{2+}$ in the myoplasm. However, until it is possible to correlate the syndrome with a specific genetic mutation in more than 90% of patients—thus allowing a simple blood test to screen for susceptibility—any less invasive test that is at least as reliable as the IVCT (CHCT) is worthy of continued evaluation.

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Safety in Numbers: How Do We Study Toxicity of Spinal Analgesics?

ADVERSE drug reactions are a common cause of injury and death in hospitalized patients. However, in many cases, serious adverse reactions are rare and are not recognized until a new drug has been in use for many years and after a huge patient exposure. Such is the case with neurotoxicity from intrathecal injection of local anesthetics; these drugs have been in use for many decades and have been given to tens of millions of patients. Nevertheless, their toxic potential is a current medical issue.

The current issue of Anesthesiology includes a report that examines the relative neurotoxicity of lidocaine and prilocaine, an alternative to lidocaine for brief spinal anesthesia. In the review process of this manuscript, concern was raised that the way the authors induced neurotoxicity (by slow infusion of drug through a small-bore intrathecal catheter with the tip deliberately placed in the cauda equina) bore no resemblance to modern clinical practice, and, therefore, the results were of questionable clinical relevance. Why did the authors do this, and are their results relevant to clinical practice? How do we go about assessing the toxicity of intrathecal drugs such as these?

Cauda equina syndrome is perhaps the most serious toxic complication of a spinal anesthetic. It is a rare event that occurs with an incidence perhaps as low as 1:100,000 following a single intrathecal injection of lidocaine. Nevertheless, considerable efforts are still being made to better understand the mechanisms of lidocaine neurotoxicity in an effort to reduce this risk. Speculation has implicated factors that include dose, concentration, hyperbaricity, and speed of injection, at least as associated with slow delivery through microspinal catheters. A common mechanism by which these factors are hypothesized to relate to toxicity is maldistribution of a drug within the thecal sac. In this, high concentrations of a drug are in prolonged contact with the sacral nerve roots, which are unprotected by sheaths. Lidocaine is of considerable concern in this regard because it has been marketed in a concentration (5%) well above that shown to be neurotoxic.

Many practitioners tacitly accept this theory of maldistribution and inject no more than 60 mg lidocaine, using nondextrose-containing nearly isobaric solutions, avoid epinephrine, dilute 5% hyperbaric lidocaine to at least 2.5% with cerebrospinal fluid or preservative-free saline, and avoid injection through a spinal catheter. Dilution of a drug before injection and avoidance of lidocaine injection through spinal catheters or small-bore spinal needles were suggested by the Food and Drug Administration in a labeling change to the 5% lidocaine formulation after 1995. Unfortunately, cases of cauda equina syndrome have also been reported after intrathecal injection of small doses of lidocaine, nondextrose-containing lidocaine, or lower concentrations of lidocaine (2%). Without knowledge of the denominator to accompany the numerator of these several case reports, one is uncertain whether the manipulations listed previously truly reduce the risk of neurotoxicity.

Returning to the current laboratory study, what are we to make of the contribution of a paradigm using the slow infusion of undiluted lidocaine to our understanding of clinical neurotoxicity? Figure 1 demonstrates the dilemma. If we assume that the incidence of serious toxicity is similar to that seen in humans, we would need to perform “routine” spinal anesthesia in nearly 300,000 animals to have a reasonable statistical likelihood of observing just one case of neurotoxicity. Ethical, financial, and practical considerations render this approach unacceptable. In other words, it is not feasible to examine this issue using a “clinically relevant” experimental protocol. Therefore, some alternative is needed. If one understood the mechanism of neurotoxicity, one might mimic local anesthetic neurotoxicity in cell culture. This is not the case with neurotoxicity from intrathecal lidocaine; we do not understand the cellular or vascular mechanism and do not know with any certainty that we can meaningfully reproduce this phenomenon in cell culture. As such, investigators, as in the current case, manipulate the in vivo experimental conditions, usually by increasing drug dose, concentration, or time of exposure, to increase the likelihood of observing toxicity in or at a more feasible incidence for study, such as 50% (fig. 1).
Does this "high-dose" approach allow us to say anything meaningful about the mechanism of a rare clinical problem that occurs following lower dose drug exposure? As one often hears from colleagues in the hallway, "What does toxicity mean if it only happens while the animals were swimming in a solution of drug?" It is broadly believed that drug effect is positively correlated with concentration. We know that lidocaine blocks sodium channels. We can devise a study to assess whether a persistent blockade of sodium channels is deleterious. Yet, it is also clear that these molecules may have a plethora of actions, with the number of target mechanisms increasing as drug concentrations increase. Therefore, at higher concentrations lidocaine may interact with membrane lipids or alter the blood–brain barrier, leading to other events. So, we may tailor the study to determine the effects of lidocaine at concentrations higher than those required to block sodium channels. Here, the issue is not whether lidocaine is a local anesthetic, but at what concentrations "other things" happen.

From a practical standpoint, the greater the separation of the sodium channel blocking concentration from the concentration that produces other effects (e.g., breaching the blood–brain barrier), the greater the therapeutic ratio and the "safer" the drug. In addition, we believe that drug effects have mechanisms that evolve over time. For local anesthetics, this time is short and the reversal of the sodium channel blockade disappears with the removal of the drug. Other effects (e.g., an effect on blood–brain barrier) may also occur short-term, but the consequences of that breaching may require days or even weeks to evolve (and to disappear). If extended exposure has no effect, the greater the likelihood that a shorter exposure will be without consequence. These lines of reasoning are consistent with the current thinking about spinal drug safety evaluation. In our animal studies of clonidine, neostigmine, and adenosine, we used drug exposures up to 100 times those used in humans up to 28 days and observed no toxicity. These observations provide reassurance that the bolus delivery of these agents at a fraction of those concentrations is likely to be clinically "safe." On the other hand, if we begin to approach those higher concentrations or change the protocol to increase the interval of exposure (e.g., alter formulation with hyperbaricity, initiate continuous infusion, or alter formulation to change drug clearance or metabolism), the rigor of our assertions of safety will be reduced. In other words, the assertion of an absence of toxicity is dependent on the conditions in which the drug was tested and those in which the drug is used.

In the present case, if one accepts the hypothesis that maldistribution is the underlying phenomenon that allows lidocaine neurotoxicity to occur, then exposing the rat spinal cord to concentrations of lidocaine no greater than those in the commercial formulation (5%) during conditions that mimic maldistribution (e.g., continued local infusion) is logical and appropriate. Importantly, the use of such a model permits comparison across drug molecules in a given class (e.g., prilocaine). If the toxicity reflects sodium channel blockade, one would predict that there would be no difference in the safety ratio defined in the rat, with continuous cauda equina infusion. On the other hand, if toxicity is dependent on another property (undefined) of the molecule that does not reflect on the sodium channel blocking properties, then it is possible that the other agent would have a different safety ratio (may be better, may be worse). This then serves to develop predictions as to possible underlying mechanisms. One may then consider the human experience (if it exists) with the drug and assess the issue of toxicity. That experience then serves to validate (or not) the preclinical model and provide insights into the underlying covariate.

One example of this regards morphine. Case reports of intrathecal granuloma concentrations have become prevalent, with a likely covariate being the use of moderate doses with low infusion rates requiring high concentrations (25–50 mg/ml). Until recently, the only systematic laboratory safety studies with intrathecal morphine were those carried out with 28-day intrathecal bolus deliveries with maximum concentrations of 10 mg/ml in dogs. These studies were specifically targeted at considering the possible safety of acute bolus delivery. Recently, we completed studies showing that in dogs high concentrations of morphine (12 mg/ml) delivered intrathecally for 28 days will also reliably result in aseptic intrathecal granulomas (personal communica-
tion between Eisenach and Yaksh, June 20, 2002). These observations (unfortunately) provide validation for the dog model and striking support for the need to examine the limits of the concentration profile in a preclinical model prior to implementation in humans.

So, how do we extrapolate the parameters of the surrogate (laboratory model) to that of the human condition? As noted, safety in humans will always initially be proposed within the context of the laboratory model in which it was studied and the assumed validity of this model. At the minimum, tissue exposure to drugs in animals should be tailored as closely as possible to that presumed to occur in humans. In the case of maldistribution of lidocaine, at least one component should include the continued infusion of the concentration of the commercial formulation itself. In this case, given concentration dependence of lidocaine-induced irreversible conduction loss in frog nerve [67], the approach used by these authors in this case follows logically from the presumed underlying factor, which allows lidocaine neurotoxicity to be manifest. Short of another few decades of human experience, this approach may be the only one to further our understanding of risk factors and mechanisms of local anesthetic-induced neurotoxicity and to hopefully improve the safety of spinal anesthesia. These considerations provide weight to the dictum of Paracelsus that there is no safe drug, only safe doses or concentrations.

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References

ANESTHESIOLOGISTS have a personal and professional interest in sleep and fatigue. Whether as a metaphor for states of anesthesia or in reference to one’s own level of arousal, the word “sleep” is a regular contributor to the vocabulary of anesthesiology. The article by Howard et al. in this issue of Anesthesiology reviews the negative influence of sleep deprivation. Projections of anesthesia work force and patient numbers continue to predict heavy workloads. Sleep deprivation causes stress, pessimism, and anger (http://www.sleepfoundation.org/nsaw/presskit.html). For anyone concerned with career sustainability, workload projections make the topic of sleep and fatigue a potentially frustrating read. Howard et al. avoid this pitfall by championing an evidence-based perspective on countermeasures that can be incorporated into one’s practice and lifestyle. Sleep disorders medicine has been embraced and advanced by pulmonology, psychiatry, and neurology. Howard et al. help explain the developing recognition in anesthesiology about the important relation between sleep and health. Opposing views that sleep deprivation is harmless are tantamount to ignoring data on the relation between smoking and cardiopulmonary disease.

"Vigilance" is part of The American Society of Anesthesiologists’ logo and the negative impact of sleep deprivation on vigilance and performance is clear from driving safety data. Motor vehicle accidents in the United States are the fifth leading cause of death, a fact that has led the American Medical Association (AMA) to endorse research and education on the risks of driving while feeling sleepy. The negative influence of sleep deprivation is so strong that the US Congress directed the Federal Highway Administration to characterize fatigue among commercial drivers. These studies found that long-haul truck drivers had less sleep than needed for alertness, averaging 5.18 h in bed and 4.78 h of sleep per day. On 27 June 2002, the House Transportation and Infrastructure Committee held a hearing on “Approaches to Improving Highway Safety.” Testimony from Darrel Drobnich, Senior Director of Government and Transportation Affairs for the National Sleep Foundation, cited statistics from The National Highway Traffic Safety Administration (NHTSA). According to Drobnich’s testimony, the NHTSA estimates that 100,000 police-reported crashes each year are the direct result of driver fatigue. These crashes cause 1,550 deaths and 71,000 injuries, as well as $12.5 billion in diminished productivity and property loss (http://www.sleepfoundation.org/PressArchives/drowsdri1testimony.html). The loss of even 1 h of sleep associated with the shift to daylight savings time has been shown to increase the number of traffic accidents. Howard et al. effectively show that the sleep and performance relation would be irrelevant only if operating a motor vehicle required greater cognitive and psychomotor skills than the safe and effective delivery of anesthesia.

Medical education is negatively influenced by sleep deprivation. Sleep enhances cortical synaptic remodeling to facilitate the memory consolidation of the waking experience. Hippocampal cell discharge patterns reflecting behavioral experiences during waking consciousness are reactivated during rapid eye movement (REM) sleep, consistent with a REM sleep-dependent role in memory processing. Objective assessment of sleep and alertness among in-house medical staff found that interns averaged less than 5 h in bed and 3.67 h of sleep per day. In a random sample of second-year residents, 25% reported being on call in the hospital more than 80 h per week and 10% reported sleep deprivation as a daily occurrence. These same residents commonly (70%) observed a colleague working in an impaired state that was most often (57% of the time) caused by lack of sleep. During the first postgraduate year, residents averaged 37.6 as the largest number of hours without sleep. Moderate sleep deprivation (17–19 h) causes impairment of cognitive and psychomotor performance equivalent to alcohol intoxication. The negative influence of sleep deprivation is so strong that under current Institutional Review Board guidelines, few institutions would approve a randomized, prospective, double-blind, controlled trial of sleep-deprived versus rested surgeons. One National Institutes of Health (NIH) database (http://www.crisp.nih.gov/) shows that questionnaire and survey data play major roles in current NIH-funded studies on "Effects of Extended Work Hours on Intern Health & Safety" (caczieszler@rics.bwh.harvard.edu) and on "Work Conditions of Surgery Residents and Quality of Care" (mentzer@pop.uky.edu). These ongoing studies and the

<!– This Editorial View accompanies the following article: Howard SK, Rosekind MR, Katz JD, Berry AJ. Fatigue in anesthesia: Implications and strategies for patient and provider safety. Anesthesiology 2002; 97:1281–94. –>
review by Howard et al.\textsuperscript{2} directly contradict minority opinions that sleep deprivation does not impair learning or performance.\textsuperscript{16}

Sleep deprivation significantly alters endocrine function, host defense, and autonomic control. In healthy young adults, sleep restriction to 4 h per night for 6 nights decreased carbohydrate tolerance, increased evening cortisol, and increased sympathetic tone.\textsuperscript{17} These endocrine changes are risk factors for development of obesity, insulin resistance, and hypertension.\textsuperscript{17} Preclinical studies show that within the first few days of sleep deprivation, normally sterile body tissues are invaded by endogenous pathogenic bacteria.\textsuperscript{18} Cytokines are known to alter central nervous system (CNS) control of sleep.\textsuperscript{19} There is evidence that bacterial and viral activation of spinal microglia and astrocytes can produce proinflammatory cytokines that amplify pain.\textsuperscript{20} There is an inverse relation between pain and sleep,\textsuperscript{21} and neural systems that evolved to regulate natural sleep states are preferentially involved in causing states of anesthesia.\textsuperscript{22}

One “hot button” topic not reviewed by Howard et al.\textsuperscript{2} is the Patient and Physician Safety and Protection Act. Representative John Conyers (D-Michigan) introduced this bill (HR 3236) in November 2001 to the 107th Congress. The HR 3236 bill proposes to reduce resident work shifts to not longer than 24 continuous h and to limit total weekly work to no more than 80 h. The bill can be viewed at http://www.thomas.loc.gov/. Sen. Jon Corzine (D-New Jersey) has introduced a companion bill to HR 3236. On June 11, 2002, while the Howard et al.\textsuperscript{2} manuscript was being reviewed, the Accreditation Council for Graduate Medical Education (ACGME) passed universal standards for resident work hours. The ACGME recommends resident work be limited to an average of 80 h per week and no more than 30 h at any one time. It also was recommended that residents be on call no more than every third night and have 1 in 7 days off from work. The complete recommendations are available in PDF format (http://www.acgme.org). In June 2002, the AMA backed the ACGME guidelines. M. Croasdale reports on the AMA position in the July 8/15, 2002 issue of American Medical News (http://www.ama-assn.org/sci-pubs/amnews/pick_02/prsb0708.htm).

Howard et al.\textsuperscript{2} signal an opportunity for anesthesiology to take a leadership role in characterizing the effects of sleep restriction and sleep deprivation on patients undergoing anesthesia, on caregivers, and on trainees. The Patient and Physician Safety and Protection Act is championed by the 40,000-member American Medical Student Association (http://www.amsa.org). Many medical students and residents are well informed about sleep neurobiology and medicine. At the University of Michigan, for example, there is a combined graduate and undergraduate course, organized from the Department of Anesthesiology entitled “Sleep: Neurobiology, Medicine, and Society.” The course is taught by faculty from departments of anesthesiology, neurology, psychiatry, pulmonary medicine, and psychology. The course attracts a significant number of allied health and premedical students. Considerable time is devoted to reviewing data on decrements in health and performance caused by overwork and sleep deprivation. All available evidence indicates that sleep, similar to breathing, is a fundamental biologic rhythm. Devaluing sleep is no longer compatible with attracting the best healthcare professionals to the specialty of anesthesiology.

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