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Indocyanine Green

Evidence of Neurotoxicity in Spinal Root Axons

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Background: **The inadvertent intravascular injection of a local anesthetic during epidural anesthesia is an uncommon but potentially serious complication. Epinephrine, the most commonly used marker, does not provide sufficient sensitivity to exclude intravascular injection in all patient populations. The dye indocyanine green (ICG) has been proposed as an alternative marker. It has been demonstrated that ICG could be used to detect intravascular injections with a simple transcutaneous spectrophotometric technique. Although the safety of intravenous ICG is well documented, its neurotoxic potential requires careful study given the probability of inadvertent intrathecal injection during test injections used to verify epidural catheter placement.**

Methods: **In this study, the authors investigated the neurophysiologic effects of clinically relevant concentrations of ICG (range, 28.6–286 μM) on single myelinated and unmyelinated dorsal root axons in rats by measuring effects on impulse generation and conduction.**

Results: **In contrast to the apparent absence of toxicity when injected intravenously, ICG applied to intact dorsal roots at concentrations likely to be encountered with an epidural test dose produced long-lasting conduction block (21 of 26 axons) or spontaneous bursting activity (7 of 26 axons) in myelinated and unmyelinated dorsal root axons.**

Conclusion: **Given this apparent neurotoxicity, ICG should not be used when intrathecal or nerve root injection is possible.**

THE inadvertent intravascular injection of a local anesthetic can result in serious central nervous system and cardiovascular toxicity, including seizures and cardiac arrest.[1–3] In a study reviewing maternal deaths in the United States from 1979 to 1990 after administration of local anesthetics during epidural or spinal anesthesia, the cause of death in 20 patients was related to possible intravascular injection or local anesthetic toxicity.[4] The incidence of inadvertent intravenous local anesthetic injection during epidural anesthesia is reported to range from 0.2 to 11% (typically 2%),[5] with an increasing incidence in pregnant patients. Various means have been proposed to test for the inadvertent intravascular placement of needles or catheters during epidural anesthesia. These techniques include the use of epinephrine,[6] isoproterenol,[7–9] air,[10] lidocaine,[11] and opioids.[12,13] The sensitivity of the most common marker, epinephrine, has been tested several times and was found to be significantly reduced in pregnant laboring women,[14] in elderly patients,[15] during general anesthesia,[16] and in the presence of β-blockers.[6] To be effective, any test must have sufficient sensitivity to permit detection of intravenous catheter placement in high-risk patient populations, thereby minimizing the risk of accidental intravenous injection. The inclusion of a readily detectable vital dye in the epidural test dose, combined with a simple noninvasive means for detecting the presence of that dye in the peripheral circulation, could prevent inadvertent intravascular injections while avoiding the problems associated with other markers.

Two studies have shown that the vital dye indocyanine green (ICG) with a maximum light absorbance between 800 and 810 nm could be easily detected by spectrophotometric techniques after intravascular doses as low as 7 μg/kg.[17,18] ICG is a water-soluble tricarbocyanine dye that in its commercial preparation (Cardio-Green®, Becton Dickinson Microbiology Systems, Cockeysville, MD) contains less than 5% sodium iodide. It has been used for 40 yr in measurements of hepatic function, liver blood flow, cardiac output, and retinal video angiography. The dye is stable in blood and is mainly bound to plasma proteins, of which β-lipoprotein is the principal carrier. It shows high hepatic clearance and undergoes no enterohepatic circulation. It has proved to be a safe compound at concentrations much higher than required for peripheral detection, but side effects have been reported. The incidence of adverse reactions to ICG at a typical dose of 500 μg/kg is very low (range, 0.05–0.2%).[19] Some of the adverse reactions appear to be related to the iodide content of the commercial preparation, and many of the reactions reported have been in patients with end-stage renal disease.[20] Neurotoxic effects have not been described. Thus, there remains some concern that inadvertent intrathecal injection during the administration of an epidural test dose could produce significant cerebrospinal fluid (CSF) concentrations of ICG that may result in a direct neurotoxic effect. The purpose of this study was to investigate the neurophysiologic effects of ICG, a proposed marker for intravascular injection, on single myelinated and unmyelinated dorsal root (DR) axons in rats by measuring effects on impulse generation and conduction.

Methods

Approval was obtained from the Stanford University Administrative Panel on Laboratory Animal Care. Sixteen

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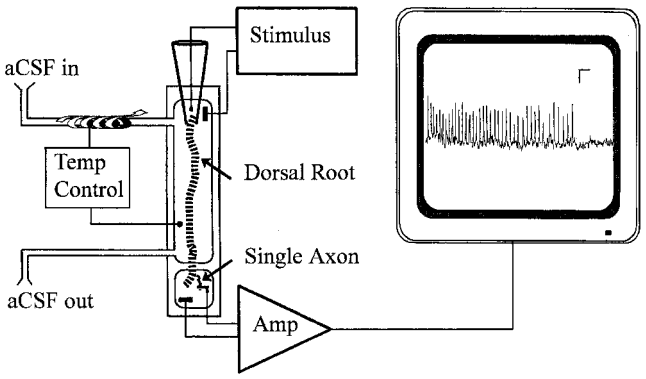

Fig. 1. Experimental setup for single-fiber recording. ACSF = artificial cerebrospinal fluid; Temp Control = automated temperature controller and monitor; Stimulus = nerve stimulator connected to a suction electrode; Amp = preamplifier and amplifier circuits connected to the computer data acquisition system. An actual example of single-fiber activity can be seen on the monitor. It was recorded from a myelinated dorsal root axon. Time (10 ms) and voltage (100 μV) calibration bars are shown in the upper right corner.

male Sprague-Dawley rats weighing 320–420 g were anesthetized with enflurane (1–2.5%) and nitrous oxide (70%) in oxygen. As previously described,[21,22] the spinal cord was exposed through a thoracolumbar laminectomy, and using microsurgical techniques, single lumbar DRs were excised and transferred to a perfusion and recording chamber. The perfusion compartment volume was approximately 1 ml, and the perfusate flow was typically 5 ml/min. The roots were continuously superfused with artificial CSF (aCSF; Na^+, 150 mM; K^+, 4 mM; Cl^-, 127 mM; Ca^{2+}, 2 mM; Mg^{2+}, 1.3 mM; PO_4^{-3}, 1.2 mM; HCO_3^-, 26 mM; glucose, 11 mM) at 37°C and equilibrated with a 95% O_2 + 5% CO_2 gas mixture to maintain a pH of 7.3–7.4. Single-fiber microdissection and recording techniques were used to isolate activity in individual spinal root axons. Dissection and recording were carried out on the proximal end of each DR after isolation from the main perfusion chamber.

Supramaximal (1.5 × threshold) constant voltage stimuli at 0.3 Hz (0.1-ms duration) were delivered to the distal end of the isolated root using a standard suction electrode. Single-fiber action potentials were amplified and displayed on a digital storage oscilloscope and recorded for computer analysis (fig. 1). Stimulus-evoked activity in individual axons was monitored, and the latency between stimulus and action potential was measured. Conduction velocity (CV) was calculated from measurements of conduction latency and length of axon between stimulating and recording electrodes (CV [m/s] = conduction distance [mm] divided by conduction latency [ms]). Control CV measurements from each axon were recorded for 20–30 min to ensure stability of the preparation before exposure to ICG (Cardio-Green®).

In a preliminary study, single ICG doses of 300 μg in 0.1 ml were injected into the constantly flowing aCSF as it entered the perfusion chamber. Each injection was repeated at 3-min intervals for a total of three injections. Based on the aCSF flow rate and injection time, we calculated that each bolus dose would transiently produce an ICG concentration ~2 times higher than the blood concentration following the standard recommended dose and ~100 times higher than the minimum transcutaneously detectable dose. This technique was designed to mimic the effects of dilution and binding believed to occur after subarachnoid injection of ICG in humans. Stimulus threshold, conduction latency, and ectopic spike generation were measured at 1-min intervals for 30 min.

In the subsequent series of experiments, isolated but otherwise intact DRs were continuously perfused with aCSF containing ICG at one of three different concentrations: group 1, 28.6 μM (n = 10); group 2, 143 μM (n = 8); and group 3, 286 μm (n = 8). These concentrations represented the 1-fold, 5-fold, and 10-fold CSF concentrations that we estimated would be present in the lumbar CSF after the inadvertent intrathecal injection of a minimum ICG test dose. This estimate was based on the following assumptions: (1) the minimum detectable intravenous ICG dose is 7 μg/kg (0.5 mg/70 kg);[17] (2) a typical test dose should contain 1 mg ICG to provide a margin of detection safety; and (3) an intrathecal injection of 1 mg ICG could equilibrate in ~50 ml of spinal CSF producing a concentration of 26 μM. However, with minimal mixing the equilibration volume could be significantly less, resulting in the exposure of spinal roots to higher ICG concentrations.

Following a series of control measurements, axons in the intact DR were exposed to the ICG containing aCSF for 25 min while repeated measurements of threshold, conduction latency, and the frequency of ectopic impulses were made. A washout period followed for about 60 min during which all measurements were continued. Only one series of measurements was obtained from one axon in each nerve root, and each axon served as its own control. Axons with unstable control measurements were not tested. As expected, the exposed portion of each intact nerve root was stained green by the ICG, and this coloration persisted during the washout period.

In a separate control study to examine the possible contribution of sodium iodide to the observed effects, single DR axons (four myelinated and two unmyelinated) were exposed to sodium iodide in aCSF at a concentration of 69 μM. This represents the approximate concentration that would be encountered with our highest ICG dose. Interval measurements were made during 25 min of sodium iodide exposure and for a 30-min washout period. Again, only one series of measurements was obtained from one axon in each nerve root.

For data analysis, DR axons were divided into two groups based on their CV characteristics. Those DR axons with a CV greater than 3 m/s were considered to be myelinated (A-fiber), whereas those with a CV less than 1.5 m/s were presumed to be unmyelinated (C-

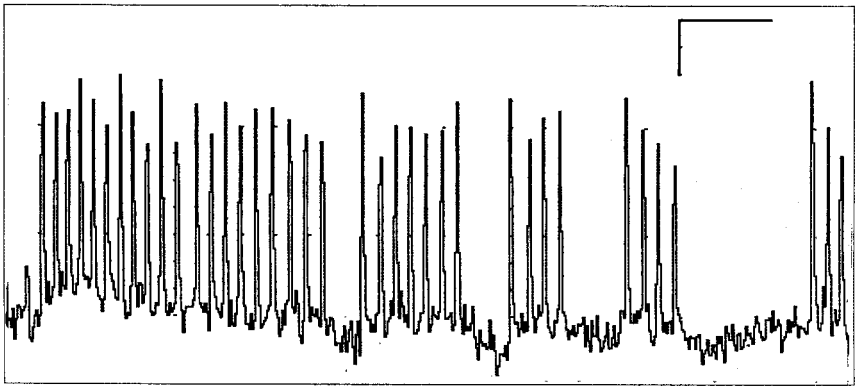

Fig. 2. Example of spontaneous bursting activity in a myelinated dorsal root axon. This activity was observed only after exposure to indocyanine green (ICG). Time (10 ms) and voltage (100 μV) calibration bars are shown on the figure.

fiber). Axons with intermediate CV were not studied. The Fisher exact text was used to measure the statistical significance of the observed differences between treatment groups. Given the small number of axons studied and the apparent absence of a significant dose–response effect, we grouped the ICG-exposed axons together for final comparison with the control group.

Results

In the preliminary experiments, exposure of isolated DRs to a series of three 300-μg bolus doses of ICG produced conduction block in two of four myelinated and two of two unmyelinated DR axons without recovery during the subsequent 30-min washout period. Spontaneous bursting activity was observed in the other two myelinated DR axons after ICG exposure (fig. 2). During the 30-min washout period this bursting activity stopped but could be started again with reexposure to ICG.

In the next series of experiments, 26 DR axons in 26 DRs from 14 rats were continuously exposed for 25 min to one of three concentrations of ICG in aCSF. The length of axon (root) exposed to ICG ranged from 17 to 26 mm. The CV for the myelinated axons ranged from 4.09 to 22.81 m/s and for the unmyelinated axons from 0.66 to 0.90 m/s. Twenty-one of these axons (81%) were affected by exposure to ICG. Conduction block occurred in 14 of 23 myelinated axons (significantly different from control), and no axon recovered from this conduction block during the 60-min postexposure observation period. The onset of block in A-fibers ranged from 8 to 50 min, averaging 23 min. In some axons, conduction block did not occur until the postexposure washout period. None of the three unmyelinated axons tested were blocked by ICG. In 5 of 23 myelinated axons and in 2 of 3 unmyelinated axons, exposure to ICG produced spontaneous bursts of action potentials. This spontaneous high frequency activity always began after ICG washout and continued throughout the postexposure period or until conduction block occurred. The incidence of conduction block and bursting activity (fig. 3) was not significantly different among the three groups but was significantly different when the grouped incidence was compared with the control. Three of 23 myelinated axons and 1 of 3 unmyelinated axons were minimally affected by ICG exposure, demonstrating only decreased CV to a mean of 62% (range, 42–77%) and 44% of control CV, respectively. CV slowing was produced by ICG in all 26 axons in an apparently dose-dependent manner (fig. 4). In many cases at the conclusion of the washout period (60 min), other axons in the intact portion of that DR were isolated and briefly studied. That was done to examine the possibility that the nerve dissection itself had somehow made the isolated axons uniquely vulnerable to ICG toxicity. However, in every DR screened in this manner, only blocked axons or axons exhibiting spontaneous bursting activity were encountered.

In the sodium iodide control study, four myelinated and two unmyelinated DR axons were exposed to a high concentration of sodium iodide (69 μM). No effects on action potential generation or CV were observed during

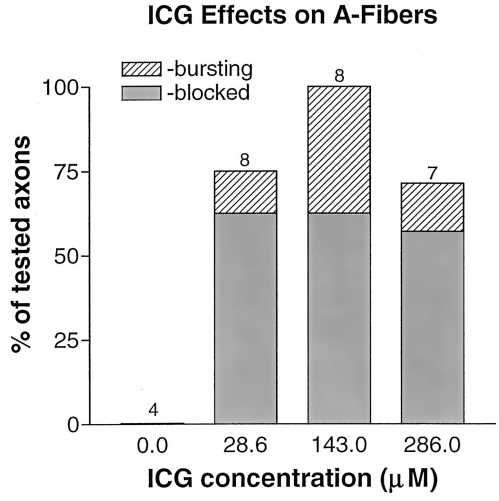

Fig. 3. Incidence of conduction block and bursting activity in dorsal root myelinated axons (A-fiber). The number above each bar indicates the total number of axons tested. Blocked fibers had complete conduction failure that could not be reversed. Axons with bursting activity displayed spontaneous episodes of high frequency action potentials.

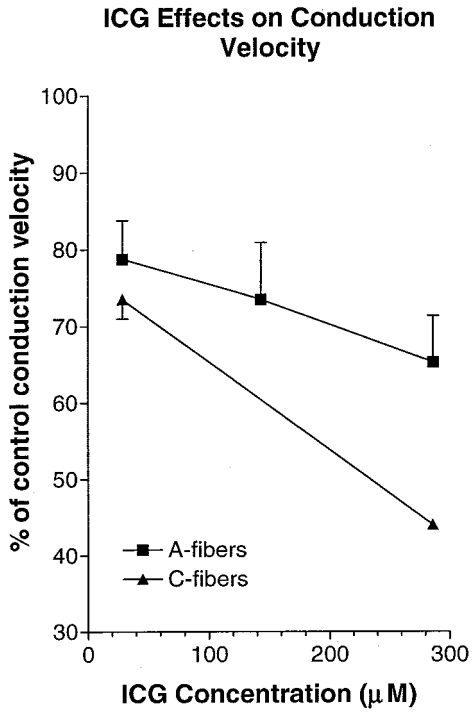

Fig. 4. Effects of indocyanine green (ICG) on conduction velocity in dorsal root myelinated (A-fiber) and unmyelinated (C-fiber) axons. Data are displayed as mean ± SD. There was no effect on the conduction velocity of axons exposed to control artificial cerebrospinal fluid (aCSF) containing 69 μM sodium iodide.

the 25-min exposure period or during the subsequent 30-min washout period.

Discussion

The results of this study demonstrate that intrathecal ICG may be potentially neurotoxic in concentrations likely to be encountered when the dye is used as a marker for inadvertent intravascular injection during epidural anesthesia. Many DR axons briefly exposed to ICG developed either persistent conduction block or spontaneous bursting activity. These effects could not be attributed to the iodide present in the commercial ICG preparation because exposure to iodide in control experiments was without effect. Clinically, intrathecal ICG could be expected to produce a combination of potentially painful paresthesias and persistent sensory loss. If motor axons in ventral roots are similarly affected, then intrathecal ICG could produce paralysis.

In a recent review of epidural injection safety procedures, the authors state that "the most significant hazard of epidural blockade is unrecognized unintentional intravascular injection,"[5] which occurs with an estimated frequency of 2% in the general population and 7–8.5% in the obstetric population. Because the consequences of bolus intravascular injection of local anesthetic can be disastrous, there is a clear need for a sensitive and specific test to detect inadvertent intravascular needle or catheter placement. Currently no such test exists. Mulroy *et al.*[5] recommend using a combination of procedures, including aspiration, incremental injections, and use of epinephrine-containing test doses in nonlaboring and other appropriately selected patients, while minimizing the concentration and dose of the local anesthetic.

The search for the perfect marker to objectively and safely detect intravascular misplacement of an epidural needle or catheter is ongoing. The dye ICG has been suggested as an alternative to commonly used markers including epinephrine.[17,18] Loehlein and Schmidt[17] were able to detect intravenously administered ICG at concentrations as low as 7 μg/kg by transcutaneous photometry at 808 nm, whereas Laurito and Chen[18] used a spectrophotometric technique at 770 nm to detect the intravenous injection of ICG at a dose of 500 μg/kg. Both groups proposed mixing ICG with local anesthetics to provide a rapid and continuous means of detecting accidental intravascular injections.

Indocyanine green is a water-soluble dye with a molecular weight of 775. After intravenous administration it is rapidly bound to plasma proteins and cannot be detected in CSF.[19] It is confined to the vascular compartment, is not metabolized, and is excreted only by the liver. Given these attributes, it has been used extensively to assess liver function, cardiac output, cerebral blood flow,[23] blood volume,[24,25] and as a contrast agent in imaging studies. ICG has an impressive safety record with relatively few adverse reactions reported since it was first approved for use in humans in 1956.[19,26] Most reactions consist of urticaria, sensations of warmth, headache, or nausea. More rarely, patients have become hypotensive or dyspneic. For example, in a series of 1,923 ICG injections, only 8 patients experienced an adverse reaction.[19] Two patients developed urticaria and were treated with oral diphenhydramine. Two others developed vasovagal-like reactions from which they recovered spontaneously. One patient became severely hypotensive and was admitted for overnight observation. The remaining three patients had mild reactions consisting of nausea or injection-related discomfort. In a review of the world literature, only eight deaths have been attributed to ICG administration, and they all appear to be the result of anaphylactic reactions.[19]

The proposal to use ICG as part of an epidural test dose to detect intravascular injection is appealing. It has many of the attributes of an ideal marker. It would be simple to incorporate into standard test doses of local anesthetic; it can be safely injected intravascularly; and its appearance in the circulation can be simply detected using a noninvasive photometric technique. However, the results of the present study have revealed a potentially neurotoxic effect of ICG on DR axons, which could preclude its use in this application.

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