Prolongation of Spinal Anesthesia

Differential Action of a Lipid Drug Carrier on Tetracaine, Lidocaine, and Procaine

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This study evaluates prolongation of spinal anesthesia by incorporating local anesthetics in lipid formulation. The duration and intensity of local anesthetic effect produced by different concentrations of procaine (1%, 2%, 4%), lidocaine (1%, 2%, 4%), or tetracaine (0.5%, 1%, 2%) dissolved in normal saline were compared to those produced by the same concentration of drugs in lipid (iophendylate) solution. Fifty rabbits with chronic indwelling subarachnoid catheters were divided into ten equal groups. Three days after the operation, the catheters were injected with aqueous solutions of the anesthetics, and 24 h later each animal received an equivalent dose of the corresponding drug in free-base form dissolved in iophendylate. The duration and intensity of motor blockade were assessed using a modified Bromage scale. A separate group of animals received plain normal saline and, 24 h later, iophendylate alone. The Kruskal-Wallis test followed by the Tukey-type test for nonparametric multiple comparisons and the Mann-Whitney and Friedman tests were used for statistical analysis at P < 0.05. Normal saline or iophendylate alone did not produce any motor blockade. Our data show that iophendylate preparations of local anesthetics produce prolonged but less intense motor blockade than the aqueous solutions, except for tetracaine 0.5% in iophendylate, which produced shorter duration of motor blockade. The reduced intensity of motor blockade may be explained by decreased availability of local anesthetic at the nerve tissue due to storage of drug in the lipid depot. The increased duration of blockade signifies a sustained release of drug from the depot. (Key words: Anesthetics, local; tetracaine; lidocaine; procaine. Anesthetic techniques, spinal; prolongation. Solvents: iodized oils; iophendylate.)

Subarachnoid administration of local anesthetics and opioids is widely used for providing surgical anesthesia, postoperative analgesia, and treatment of chronic pain. A single injection of local anesthetic into the epidural or intrathecal space has a limited duration of action. Catheterization techniques have been developed to prolong anesthetic effect. Continuous catheterization of the epidural space has been in vogue for a long time, and recently catheterization of the intrathecal space has been gaining popularity. However, complications associated with epidural and spinal catheterization techniques have been described. These include breakage of the catheter, inadvertent intravascular injection of the drug, migration of the catheter subdurally, toxicity related to high plasma levels of the drug as result of repeated administrations, and cauda equine syndrome. Moreover, chronic histopathologic changes attributed to the presence of the catheter in the spinal space have been noted to occur in the spinal cord region in close to the tip of the catheter after intrathecal infusion of plain normal saline. Thus, in order to provide prolonged local anesthesia or analgesia, it may be desirable to develop a slow-release formulation of local anesthetics, which in some cases may obviate the need for a catheterization technique.

Over the past decade, investigators have attempted to implement slow-delivery drug systems in anesthesiology. Liposomes have been shown to prolong epidural anesthesia produced by bupivacaine or lidocaine and topical anesthesia by tetracaine. Lecithin-coated methoxyflurane microdroplets have been shown to produce prolongation of sensory anesthesia when applied directly to the skin. A biodegradable polymer was recently reported to prolong sciatic nerve blockade produced by bupivacaine. In our previous studies, we have shown that slow-release formulations using a lipid vehicle may prolong spinal anesthesia and analgesia. The present study evaluates the action of different concentrations of lidocaine, bupivacaine, and procaine injected into the subarachnoid space in an iophendylate slow-delivery system.

Materials and Methods

Experiments were performed in accordance with the established guidelines and were approved by the Institutional Committee of Animal Care. Fifty Dutch Belt rabbits weighing 2–2.5 kg were housed individually in standard 40 × 50 × 50 cm cages with a 12-h light–dark cycle. The animals were provided with free access to food and water. A detailed description of chronic catheterization of the subarachnoid space has been provided elsewhere. Briefly, polyethylene tubing (PE 10, Clay Adams, Parsippany, NJ), 300 mm long, OD 0.61 mm, and ID 0.28 mm was inserted in the subarachnoid space under sterile conditions. A nylon monofilament suture 6-0 was tied around the catheter 30 mm from the proximal end and secured with epoxy glue. Each catheter was sterilized with 70% alcohol and prior to implantation irrigated with sterile.
water. The open outer tip was closed with a sterile stainless steel plug.

Rabbits were premedicated with droperidol 1 mg/kg, atropine 0.1 mg/kg, and ketamine 50 mg/kg. A marginal ear vein was cannulated, and ketamine was infused continuously 1 mg·kg⁻¹·min⁻¹. Cefazolin 50 mg/kg was injected subcutaneously for infection prophylaxis. Three milliliters 2% lidocaine was injected subcutaneously between the sixth lumbar and first sacral processes. The seventh lumbar spinal process, ligamentum flavum, and epidural fat were removed exposing the dura and spinal cord. A slit was made through the dura and arachnoid membranes, and the distal end of the catheter was inserted into the subarachnoid space in a caudal direction and secured to the peristemeum of the posterior lamina. The free outer end was placed into a subcutaneous tunnel, exteriorized in the dorsal cervical region and secured to a subcutaneously implanted button.

DRUGS AND INJECTIONS

All drug solutions were prepared on the day of the experiment. Control solutions were prepared by dissolving tetracaine, procaine, or lidocaine hydrochloride salts in preservative-free normal saline, and test solutions were prepared by dissolving an equimolar mass of the free-base form of each of these drugs with iophendylate (Pantopaque®, Alcon Laboratories, Humaco, Puerto Rico). Pantopaque® is prepared by esterification with ethyl alcohol of the mixture of acids obtained by direct iodination of phenylundecanoic acid. It contains 30.5% of organically bound iodine and has a specific gravity 1.28 at 20°C. No preservative was used in the solute or the solvent of any preparations.

All injections were performed at least 72 h after surgery. In each instance the injected volume was 50 μl/kg and injected via the subarachnoid catheter at a rate of 0.25 ml/min with a 0.25-ml syringe fitted with a 30-G needle. An infusion pump (Harvard Apparatus, Inc., South Natick, MA) was used to ensure a constant rate of injection. After administration of the drug, the catheter dead space (15–18 μl) was flushed with 40 μl normal saline. First, the saline preparation was administered and then, 24 h later, the lipid preparation was given. This order of injections was used to avoid influences of iophendylate, which remains in cerebrospinal fluid (CSF) close to the site of injection for a long time. (We observed the phenomenon of considerable prolongation of effect produced by tetracaine injected 24 h after injection of iophendylate in comparison with administration of the drug into intact animals. Therefore, animals that received iophendylate once were not used in further studies, and the order of aqueous/lipid solution could not be randomized). After the selection of the appropriate concentrations, aqueous preparations were administered randomly.

Reproducible effect and absence of tolerance after repeated and continuous intrathecal administration of local anesthetics has been documented in dogs up to 16 weeks and recently has been reported in humans. In our previous study, we demonstrated a reproducible effect after repeated spinal administration of local anesthetic up to 12–14 days in the rabbit model. Therefore, in the current study, each animal was used as its own control.

To compare the in vitro rate release of the drug from the lipid vehicle with drug diffusion from a hyperbaric aqueous vehicle, we used solutions of the anesthetic’s base forms in iophendylate and of its hydrochloride forms in 50% glucose, respectively. Aqueous solution was prepared using 0.15 M HEPES (Sigma, St. Louis, MO) as a buffer and adjusted to a pH of 7.4 with NaOH. Three hundred milliliters of solution was placed on a stirrer (7-SH, Fisher Scientific, Pittsburgh, PA) in a beaker with an internal diameter of 90 mm. The stirrer was angled 5° to keep the hyperbaric solution as a whole mass and to prevent its spread over the bottom of the glass. A stirrer bar of 8 mm in length was placed in the middle of the beaker bottom. The speed of rotation was adjusted to maintain a uniform concentration of released drug in the buffer solution and not to mix the hyperbaric solutions with the buffer. Solutions of the anesthetics in iophendylate were prepared in a concentration of 0.4%, and equimolar aqueous solutions were prepared in 50% glucose. Three milliliters of hyperbaric solution was sunk to the lowest part of the beaker bottom. Two-milliliter samples of solution were taken for evaluation of the drug concentration and were returned into the beaker after each measurement. Assessments of the concentration were carried out using ultraviolet spectrophotometry (UV 160 U, Shimadzu, Japan). Determination of absorbance was performed at 318, 225, and 313.5 nm for procaine, lidocaine, and tetracaine, respectively.

Pilot studies were performed to find the range of drug concentrations able to produce effect in both aqueous and lipid preparations. In our previous experience, 2% procaine or lidocaine or 1% tetracaine in saline produced noticeable motor blockade in rabbits. For each anesthetic, additional concentrations, which were 100% greater or 50% less than the initial concentration, were tested. This order was used for further selection of the appropriate concentrations.

Finally, concentrations of procaine (1%, 2%, 4%), lidocaine (1%, 2%, 4%), or tetracaine (0.5%, 1%, 2%) were selected. For each concentration, 5 rabbits were used, yielding a total of 45 for the three anesthetics. An additional 5 rabbits were used to assess the motor effects of normal saline and iophendylate.

Each animal was tested with only one concentration of one local anesthetic in both solvents. Injections of the drug and evaluation of the effect were performed by dif-
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Evaluation of the Pharmacologic Effects

The anesthetic action was evaluated by the intensity of motor block using Bromage et al.'s scale for humans, modified for the rabbit model as follows:

0 = free movements using hind limbs without limitations
1 = limited or asymmetrical movements of the hind limbs to support the body and walk
2 = inability to support the back of the body on the hind limbs, with detectable ability to move the limbs and respond to pain stimulus (Paw pinch)
3 = total paralysis of the hind limbs

The animals were taken out of the restraining hammocks and put on the floor at 10-min intervals after injections, and an attempt was made to walk them.

Intensities of motor blockade were represented by the median, and statistical analysis, using the Fisher exact test, was applied. Durations of motor blockade and times to achieve peak intensity were computed and analyzed by means. The Kruskal-Wallis test followed by a nonparametric Tukey-type multiple-comparisons test and the Mann-Whitney test, when appropriate, were used for analysis of the differences among the groups. The Friedman test was used to compare the parameters between saline and iophendylate preparations within the same animal group.

Results

All animals recovered from anesthesia, and none developed postoperative neurologic deficit or wound infection. No animal showed evidence of discomfort such as writhing or squeaking during injection, and no animal developed permanent hind limb paralysis. Rabbits that received only saline or iophendylate did not develop motor block. Complete data collection was not possible in animals receiving 2% tetracaine in lipid solution because beyond 7 h the rabbits failed to ambulate, most likely because of fatigue of the fore limbs as a result of repeated testing; consequently, they were not included for statistical analysis.

Intensities and durations of motor blockade are presented in figure 1. The actual duration of motor block as well as the ratios between the durations of motor block produced by the two preparations are given in figure 2.

Motor block reached peak intensity within 5 min after injection of the aqueous preparation and 5–15 min after injection of the lipid preparation. The lipid preparations containing 1% procaine, 1% lidocaine, and 0.5% or 1% tetracaine took significantly longer to achieve peak effect than the respective saline preparations ($P < 0.05$ for all three preparations).

Aqueous solution of 4% procaine produced stage-3 motor block in three animals and stage-2 motor block in two animals. 2% aqueous solution produced second-degree blockade in all animals. Three of five animals treated by 1% procaine exhibited stage-2 motor blockade, and two animals displayed stage-3 blockade. Differences among intensities produced by different concentrations of aqueous solutions of procaine did not reach statistical significance. Lipid solution of 4% procaine produced stage-2 blockade in all animals, and solution of 1% produced stage-1 blockade. Lipid solution of 2% procaine...
Aqueous solution of 2% lidocaine produced motor blockade of stages 2 and 3 in two and three animals, respectively. Solution of 1% lidocaine produced stage-2 block in all animals, and solution of 0.5% produced stage-1 block. Lipid solution of 2% lidocaine produced stage-2 motor block in all animals, and solution of 1% produced stage-2 block in four animals and stage-1 block in one animal. Lipid solution of 0.5% lidocaine did not produce a motor blockade. The intensities of motor blockade produced by 1 and 2% aqueous lidocaine were significantly greater than the intensity produced by the 0.5% solution ($P < 0.004$ and $P < 0.001$, respectively). Differences between the 1 and 2% solutions, either aqueous or lipid, were not significant.

Aqueous solutions of tetracaine produced stage-3 motor block in all animals for all three concentrations tested. Lipid solution of the anesthetic produced second-degree blockade for 1 and 2% concentrations, which was significantly greater ($P < 0.001$) than the effect produced by the 0.5% solution of tetracaine. Lipid solution of 0.5% tetracaine produced stage-1 motor block in four of five animals; one animal did not exhibit motor blockade.

At equivalent concentrations, all three aqueous solutions of tetracaine produced greater peak effect than the respective lipid preparations (fig. 1). When the peak effects were compared between aqueous and lipid preparations of procaine and lidocaine, none of the differences reached statistical significance.

The duration of motor blockade increased with increasing dose of each local anesthetic for lipid as well as for aqueous preparation (fig. 1). The durations of motor blockade produced by aqueous solutions of 2% and 4% procaine were greater than the duration produced by 1% procaine ($P < 0.05$ and $P < 0.01$ respectively). The duration produced by the lipid preparation of 4% procaine was greater than the durations produced by 1% and 2% solutions ($P < 0.01$ for both comparisons). The durations of motor block produced by 1% and 2% concentrations of aqueous lidocaine were significantly greater than the duration produced by 0.5% solution ($P < 0.05$ and $P < 0.01$ respectively). The comparison between the durations of the block produced by the lipid preparations of 0.5% and 1% showed significant difference ($P < 0.01$). The durations of the motor blockade produced by the aqueous and lipid preparations of tetracaine increased with increase of the dose ($P < 0.01$ for all comparisons among the groups). All lipid preparations produced significantly longer motor block compared to their aqueous counterparts except 0.5% tetracaine.

Concentrations of the local anesthetics obtained from the buffer solution during the time of drug release from the iophendylate and glucose solutions are presented in figure 3. All local anesthetics dissolved in glucose reached maximum absorbance within 5 min after sinking solutions.
in the beaker. The concentrations of procaine, lidocaine, and tetracaine released from the lipid solution did not reach maximum absorbance within 10 h.

**Discussion**

Our data show that local anesthetics carried in a lipid vehicle produce spinal anesthesia of long duration. The differential solubility of the ionized and nonionized species in aqueous and lipid solution was the basis of slow-delivery system. The ionized hydrochloride salt was used to prepare the aqueous formulation, and the lipophilic unionized base was used to prepare the lipid formulation. Since the base form of most local anesthetics is insoluble in aqueous solution, after subarachnoid injection of the lipid drug formulation, it remains within the lipid depot. Prior to diffusion into the CSF, the lipid-soluble unionized form of the drug undergoes conversion to the ionized water-soluble form. Thus, our speculation is that the ratio of the degree of ionization, which depends on the pKa of the drug and the pH of the CSF, determine the amount of drug available for diffusion.

Three important kinetic processes determine the local anesthetic concentration at nerve tissue after intrathecal administration: 1) diffusion of the drug from the injected solution into the CSF; 2) uptake by nerve tissue; and 3) elimination of the drug from CSF and nerve tissue by blood flow. The first and second processes determine the onset, spread, and intensity of the block, and the third process determines the duration of the drug action. Depending on the relationships among rate constants and drug properties, different groups of drugs show different patterns of action. For example, hydrophilic morphine displays delayed onset and prolonged duration of action in comparison with lipophilic sufentanil. This occurs because, in pharmacokinetics of spinal narcotics, the rate-limited process is the uptake of the drug from the CSF. In contrast, uptake of local anesthetics from the CSF is a fast process. Therefore, the duration of their action will depend on drug elimination from the nerve tissue. Exceedingly strong partitioning into membrane structures probably explains the long-duration blocks from lipophilic anesthetics. As a result, lipophilic tetracaine displays a block of greater duration than does hydrophilic procaine.

To produce effect, the amount of drug released from the vehicle must be sufficient to maintain a certain concentration of anesthetic on the nerve tissue. To obtain prolongation of effect, the rate release constant of the anesthetic from the lipid phase must be less than the elimination rate constant of the drug from the nerve cells. If drug concentration declines less then the effective level, a reduction or cessation of the effect may occur. For example, 0.5% tetracaine in lipid preparation produced shorter motor blockade compared to aqueous solution, and the lipid preparation of 0.5% lidocaine did not produce an effect. An increase in concentration resulted in significant prolongation of the effect produced by lipid preparations of both drugs compared to their aqueous solutions.

We chose to use motor blockade as a marker of local anesthetic effect because accurate evaluation of sensory blockade is difficult in a rabbit. The most widely used methods of testing nociception in rats and mice—tail flick and hot plate—are not applicable to the rabbit. Other methods, such as electrical stimulation, thermal probe stimulation, and evoked potentials, require prolonged restraining of experimental animals. As we previously reported, restraining rabbits longer than 2 h causes agitation and affects the evaluation of pain threshold, most likely because of the overfilling of the urinary bladder (rabbits are unable to urinate in a restrainer).

Motor and sensory impulses are transmitted by different nerve fibers; however, the molecular mechanism of local anesthetic action is the same regardless of fiber type. Moreover, similar profiles of motor and sensory blockade obtained after intrathecal administration of local anesthetics have been reported. Hence, motor blockade has been used by investigators to evaluate spinal anesthesia in animal models and humans. According to our results, the most effective among the tested drugs in obtaining prolongation of anesthetic action was tetracaine. Lipid solutions of 4% procaine, 2% lidocaine, and 1% tetracaine reached the same degree of intensity of motor blockade. However, durations of the motor blockade produced by these drugs were 68 ± 6, 71 ± 4, and 348 ± 34 min, respectively (fig. 4). It most likely occurs because of the strong lipophilicity of tetracaine due to a higher partition coefficient in comparison with
lidocaine or procaine. This suggests that, if a prolonged duration of action is to be achieved with a lipid vehicle, use of a drug with high lipid solubility may be beneficial.

Our in vitro experiments clearly showed sustained release of the drug from the lipid solution, exhibited by all three local anesthetics (fig. 3). Procaine and lidocaine were completely diffused into CSF within 10 h. The concentration of tetracaine in buffer solution showed that after 24 h 59% of the drug remained in the iophendylate. We believe that such results support our hypothesis that prolongation of the anesthesia produced by the lipid solutions of anesthetics is based upon a slow-release mechanism. The duration values of motor blockade produced by both aqueous and lipid solutions of 1% tetracaine obtained in the current study showed some difference in comparison with the results from our previous publication. Durations of the motor blockade in this study was 115 ± 7.9 and 348 ± 34 min versus 130 ± 7.0 and 447 ± 13 in our previous study for aqueous and lipid solutions, respectively. We believe that this variation was due to differences in the strain and weight of experimental animals. The weight of the rabbits used in our previous study ranged from 3–3.5 kg versus 2–2.5 kg in the current study.

Several questions remain unanswered concerning the optimal solvent for lipid formulations. We chose iophendylate because it has been used extensively in humans for myelography. However, aseptic arachnoiditis was reported to occur after intrathecal injection of iophendylate (Pantopaque). Therefore, use of this drug as a vehicle in clinical practice is controversial. Development of appropriate lipid solvents and evaluation of their effect upon the spinal cord may be required.

References

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Fig. 4. Motor blockade produced by lipid solutions of procaine, lidocaine, and tetracaine in concentrations that reached the same degree of the block.


