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Microdialysis and Delivery of Iontophoresis-Driven Lidocaine Into the Human Gastrocnemius Muscle

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Context: Iontophoresis is used frequently in physical medicine and rehabilitation, but many research techniques do not adequately measure it for depth of medicine delivery.

Objective: To determine if iontophoresis delivers lidocaine 5 mm under the surface of human skin.

Design: Descriptive laboratory study.

Setting: Therapeutic modalities research laboratory.

Patients or Other Participants: Eight men and 5 women volunteers (age range = 21 ± 2.3 years) who had less than 5 mm of adipose tissue in the area we measured participated in the study.

Intervention(s): We inserted a microdialysis probe 5 mm under the skin of both legs and into the triceps surae muscle groups of 10 participants. Microdialysis was performed for 60 minutes to allow a recovery period for local skin blood flow to return to baseline. We then delivered 2 mL of 1% lidocaine to the treatment leg via iontophoresis at 40 mA/min. Next, microdialysis was performed continuously in both legs during the treatment and for 30 minutes posttreatment to collect the lidocaine samples. After we had gathered the samples, several saline solutions with various amounts of lidocaine (0.005%, 0.025%, 0.05%, and 0.1%) were prepared in vitro and analyzed. Although we did not intend to do so as a part of the original study, we also performed an identical follow-up study at 3 mm in 3 participants.

Main Outcome Measure(s): Both in vitro and in vivo samples were analyzed via reverse-phase high-performance liquid chromatography (RP-HPLC). A protocol for detection and quantification of lidocaine using RP-HPLC was followed.

Results: We did not detect any measurable levels or concentrations of lidocaine in the 10 control samples. According to the RP-HPLC analysis, the 10 treatment samples also were negative for the presence of lidocaine. However, when we performed the study at 3 mm, microdialysis detected lidocaine in the 3 participants at this depth in the treatment leg only.

Conclusions: Measurable levels of lidocaine were not detected at 5 mm but were found at 3 mm. More studies are needed to determine the efficacy of microdialysis in measuring iontophoresis-delivered compounds.

Key Words: drug delivery, electricity

Key Points

- Microdialysis did not detect 1% lidocaine at a depth of 5 mm in the participants' legs.
- Microdialysis is an appropriate method to use to determine and quantify the presence of 1% lidocaine in the human calf at a depth of 3 mm when delivered via iontophoresis.

Therapeutic drug use can have undesirable side effects, such as organ and tissue damage due to oral medication or pain, infection, and scarring due to needle injection. Iontophoresis was developed nearly 50 years ago as a less painful way to deliver medication to a specific area via electrodes.[1] The use of iontophoresis for transdermal drug delivery is common.[2–6] In physical medicine and rehabilitation, the most commonly used drugs are acetate, dexamethasone, hydrocortisone, and lidocaine. Traditionally, the dose used to bring about the desired effects has been 40 mA/min.

The principle behind iontophoresis involves using electric repulsion to drive an ionic compound through the skin by placing a like-charged electrode over the compound.[3] Although still contested, researchers think the ionic compound follows the electric current into the skin using pores (especially of the sweat glands) as pathways. The movement of the compound beyond the subcutaneous layers is thought to occur as a result of blood flow and diffusion.[3,7–9]

However, some researchers have doubts about the success of this method in delivering medication to the desired treatment area. One major concern hinges on the ability of the medication to penetrate the skin and reach the targeted tissue.[9–13] The mixed results from previous research might be attributed in part to the many different treatment variables used (time, dose, electrode size) and the various methods of collecting and analyzing the drug compound.[9,10]

Microdialysis is a technique that uses the *dialysis* (from Greek, meaning to separate) principle, by which a probe that is permeable to water and small solutes is inserted into the tissue to collect or sample various compounds. The probe is perfused with a liquid (typically saline) that equilibrates with the fluid outside its membrane by diffusion in both directions. This

method allows the extracellular fluid (ECF) composition and response to exogenous agents to be observed and analyzed.[14,15] It includes the sampling of ECF, either to assess the concentration of local chemical compounds or to perfuse drugs directly into small clusters of cells.

Microdialysis is a semi-invasive sampling technique that is used in preclinical and clinical pharmacokinetic studies for continuous measurement of free, protein-unbound concentrations in extracellular tissue fluids by means of a microdialysis catheter (or probe). The microdialysis probe consists of a semipermeable hollow-fiber membrane that is perfused constantly with a solution (perfusate) at a low rate of approximately 0.1 to 5 μL/min. After insertion into the tissue or the body fluid of interest, small molecules can cross the semipermeable membrane by passive diffusion. The microdialysis principle was first used in the early 1960s to study biochemistry in animal tissues, especially rodent brains.[1] During the 1970s, the microdialysis catheter was improved greatly and eventually resulted in today's most prevalent shape, the needle probe.[2]

During microdialysis, molecules in the tissues diffuse into the perfusate as it is pumped slowly through the microdialysis probe. The dialysate then is collected and analyzed to determine the identities and concentrations of molecules that were in the ECF. The concentration in the dialysate of any given substance normally is much lower than the concentration present in the ECF, especially for substances with a relatively high molecular weight. Typically, the concentration of a peptide collected by microdialysis is just 5% to 10% of the original concentration. This depends on the charge and size of the molecule in question and on the dialysis speed.

Microdialysis has been adopted for studies in man to investigate free concentrations of various substances in the extravascular and extracellular spaces.[16] It has been used to study dopamine neurotransmission[17] in the injured human brain.[16,18] Microdialysis also is commonly used to deliver drugs to organs, to measure blood flow, and to measure the rate of sweating.[19] Currently, neuroscientists use microdialysis to study the release of neurotransmitters in the brain.[17]

Regardless of the use, the desired solution needs to be analyzed after it is collected. One accurate and objective measure of the compound of interest can be performed using reverse-phase high-performance liquid chromatography (RP-HPLC). This process is used to detect and quantify the amount of a compound in liquid solution. It accomplishes this by separating molecules in a liquid solution based on their various levels of hydrophobicity. The RP-HPLC method has been shown[17] to represent both a reliable and valid way to detect and quantify lidocaine in solution.

Therefore, the purpose of our study was to determine if microdialysis could recover lidocaine in subcutaneous tissue during iontophoretic delivery. We hypothesized that microdialysis followed by RP-HPLC analysis would be an effective method by which to not only detect, but also quantify, the amount of 1% lidocaine delivered during an iontophoresis treatment.

METHODS

In Vitro Drug Delivery

Before participant recruitment and data collection, we performed several in vitro experiments. These "bench-top" experiments were conducted for the following reasons: (1) to ensure that if the lidocaine penetrated to the depth of the microdialysis probe, the drug could be collected and recovered by the probe; (2) to assess the possibility of protein binding blocking the recovery of lidocaine via the microdialysis probe; and (3) to develop a linear relationship between percentage of lidocaine administered and the amount recovered.

During the first bench-top experiment, a microdialysis probe was connected to an infusion pump (model Pump 11 VPF; Harvard Apparatus, Holliston, MA) and placed in a petri dish filled with saline solution (0.9% NaCl). A collecting vial (1.5-mL microcentrifuge, Safe-Lock tube; Eppendorf, Hamburg, Germany) was placed at the opposite end of the probe to collect the diasylate during infusion. Two milliliters of 10 mg/mL lidocaine was added directly to the saline solution in the petri dish. During collection, saline solution (0.9% NaCl) was pumped through the probe at a constant rate of 0.011 mL/min and collected in the vial for analysis. This was repeated several times with various concentrations in separate petri dishes. The different samples were analyzed using RP-HPLC to detect and quantify the presence of lidocaine. A previously established RP-HPLC protocol[17] for detection and quantification of lidocaine was followed. The results revealed that the microdialysis probe could recover lidocaine and that the RP-HPLC analysis could detect lidocaine concentrations equal to or greater than 0.05 mg/mL.

The second series of bench-top experiments involved procedures similar to the first. However, this time blood plasma also was added to the saline solution in the petri dish to assess the possible effects of protein binding on the recovery of lidocaine. In the absence of blood plasma, microdialysis recovered approximately 6.5 mg/mL of the 1% lidocaine that was administered. With the presence of plasma in the solution, microdialysis recovered only 1.6 mg/mL. Although the high concentration of blood plasma had a huge effect on the ability of the microdialysis probe to recover the lidocaine, it did not completely block recovery (Figure 1).

The last in vitro experiment involved diluting the treatment solution of lidocaine to various concentrations (1, 0.5, 0.25, and 0.05 mg/mL) with saline. This was done for 2 reasons. First, we wanted to have an idea of how sensitive the RP-HPLC analysis was. Second, we wanted to generate a standard curve, which would allow us to quantify the amount of lidocaine detected in the subsequent samples. The RP-HPLC analysis was able to detect the presence of lidocaine at the 0.05-mg/mL concentration. A line of best fit was generated from the standard curve ($y = 155615x - 76.687$, $R^2 = 0.9999$), allowing us to determine the linear relationship between the area under lidocaine's RP-HPLC peak and percentage of lidocaine (Figure 2). With this linear relationship established and the success of our in vitro experiments, we were confident that microdialysis in conjunction with RP-HPLC analysis could detect and quantify the presence of lidocaine in a subcutaneous environment.

Participants

Participants, whom we recruited from exercise science classes, reported to the University Human Performance Research Center for screening. Skinfold measures of the calf were taken. The skin was folded during the measurement, and participants were excluded from the study if they had more than 10 mm of skinfold thickness (ie, less than 5 mm of cutaneous tissue).

Other inclusion criteria included the following: area free of any injury, swelling, or infection for at least 3 months before the study and no allergies to lidocaine. The first 13 participants

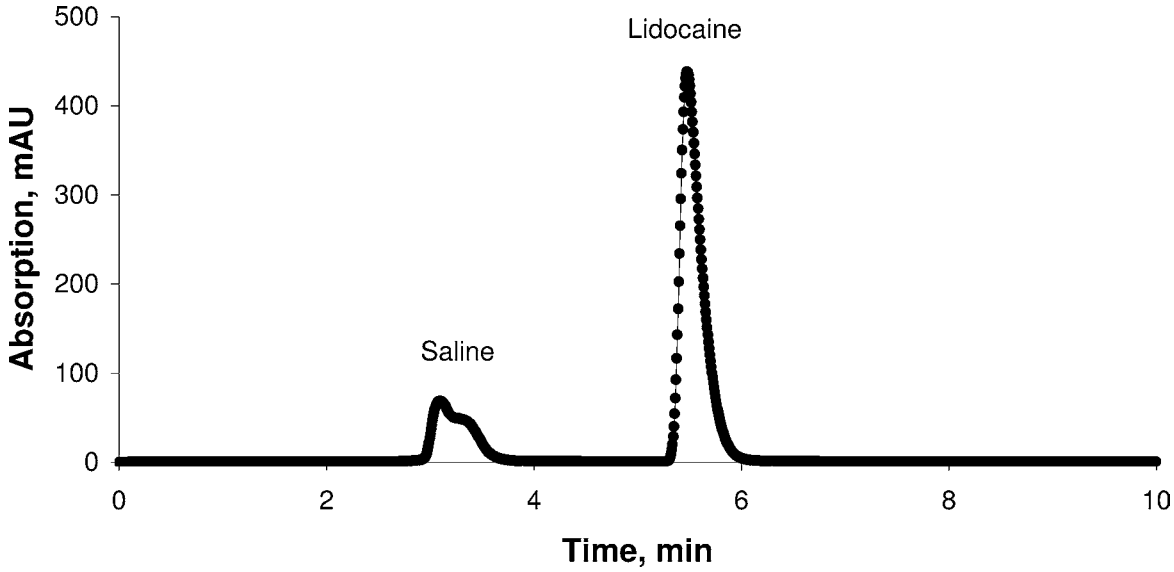

Figure 1. High-performance liquid chromatography analysis profile of in vitro results in high plasma concentration.

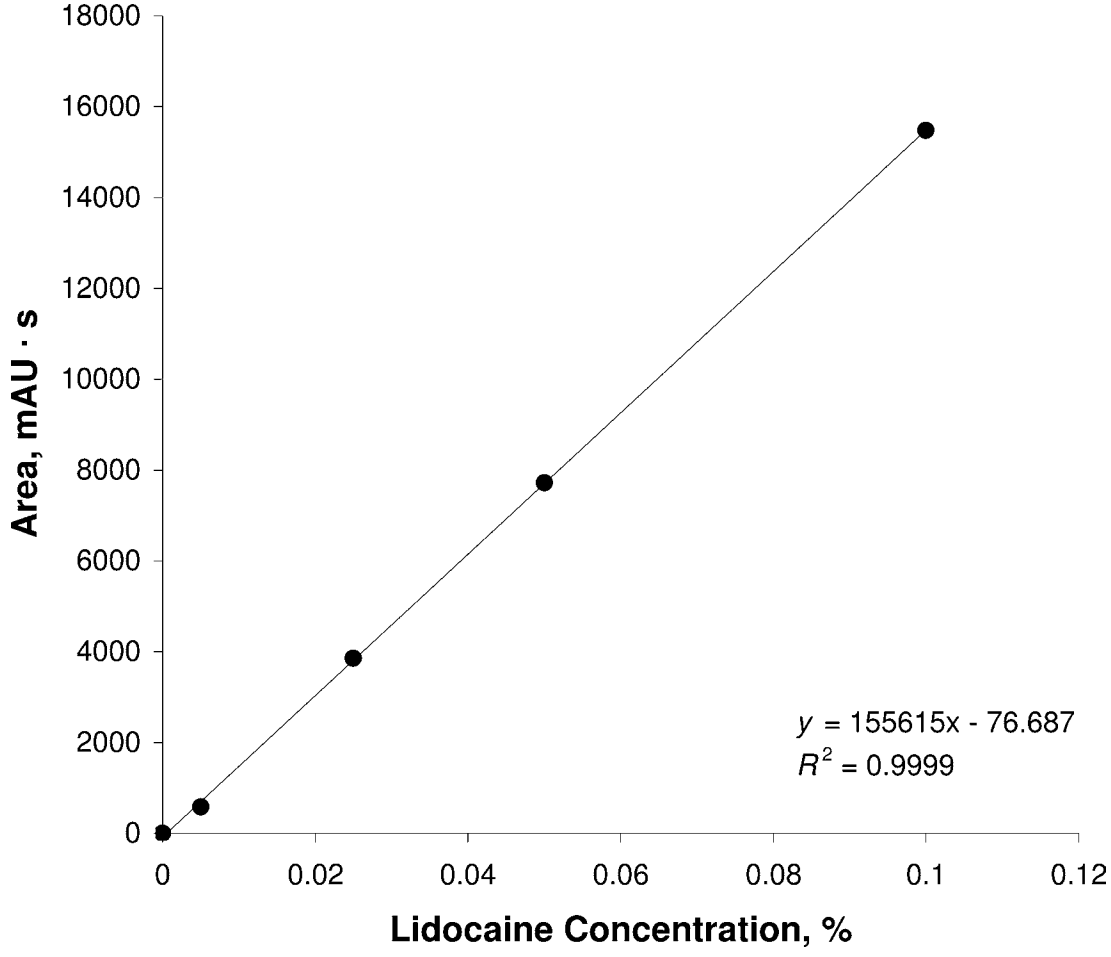

Figure 2. Standard curve with line of best fit.

(8 men, 5 women; age range = 21 ± 2.3 years) who met our criteria were included in the study; 10 were involved in the originally planned portion of the study, and 3 were involved in an unplanned follow-up portion of the study. Participants provided written informed consent, and the study was approved by the institutional review board at Brigham Young University.

Instruments

An iontophoresis device (ActivaTek, Salt Lake City, UT) was used to deliver the lidocaine. One electrode carried the drug to the treatment site, and one electrode served as the dispersive electrode (both electrodes: Trivarion; ActivaTek). The infusion of the microdialysis probe was performed using an infusion pump (model Pump 11 VPF; Harvard Apparatus). The microdialysis probes were manufactured in our university's laboratory (Figure 3).

Probe Placement. Each probe was gas sterilized with ethylene oxide before the study. Sterile technique was followed throughout data collection for all participants. The treatment site was cleaned with a povidone-iodine swab and an alcohol preparation wipe before insertion of a 27-gauge spinal needle. A microdialysis probe was inserted 5 mm below the surface of the skin of both legs (at the site of greatest calf girth) using the 27-gauge spinal needle as a guide cannula for 10 participants and, during the follow-up portion of the study, 3 mm below the skin's surface for 3 participants. The entrance and exit sites of the skin were separated by 5 cm to allow adequate spacing for the treatment electrode's reservoir. After feeding the probe through the spinal needle, we removed the cannula, leaving the probe in place. Before removal of the spinal needle, diagnostic ultrasound (LOGIQ P6; GE Healthcare, Wauwatosa, WI) was used to verify the depth of the guide cannula. After placement of the microdialysis probe, infusion was performed for 60 minutes at a constant rate of 0.011 mL/min to allow for a recovery period. This allowed local skin blood flow to return to near-normal levels after tissue irritation due to probe placement.

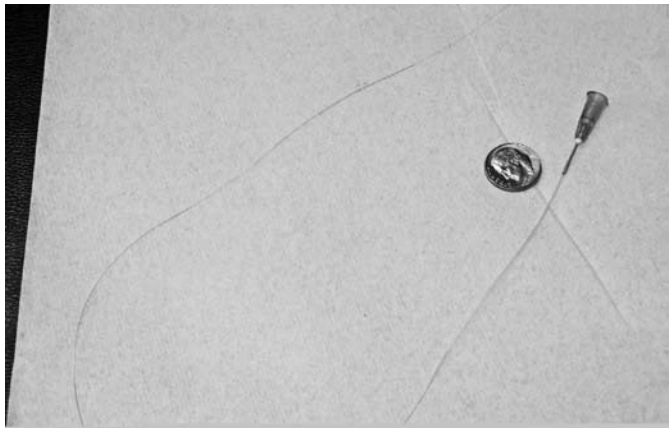

Figure 3. The microdialysis probe used to collect the drug compound.

Drug Delivery Treatment In Vivo. After probe insertion and the recovery period, the infusion rate was changed to match the rate of our in vitro experiments (0.0055 mL/min), and a new collecting vial was connected to the probe. The drug-delivery electrode (positive) was prepared with 2 mL of 1% lidocaine (positive charge) and placed on the treatment site directly over the microdialysis probe. The 14-cm^2 delivery electrode had a 2.0-mL reservoir. The larger (37-cm^2) dispersive electrode was placed 6 in (15.2 cm) proximal to the drug-delivery electrode. After the electrode leads were secured, the phoresor was set

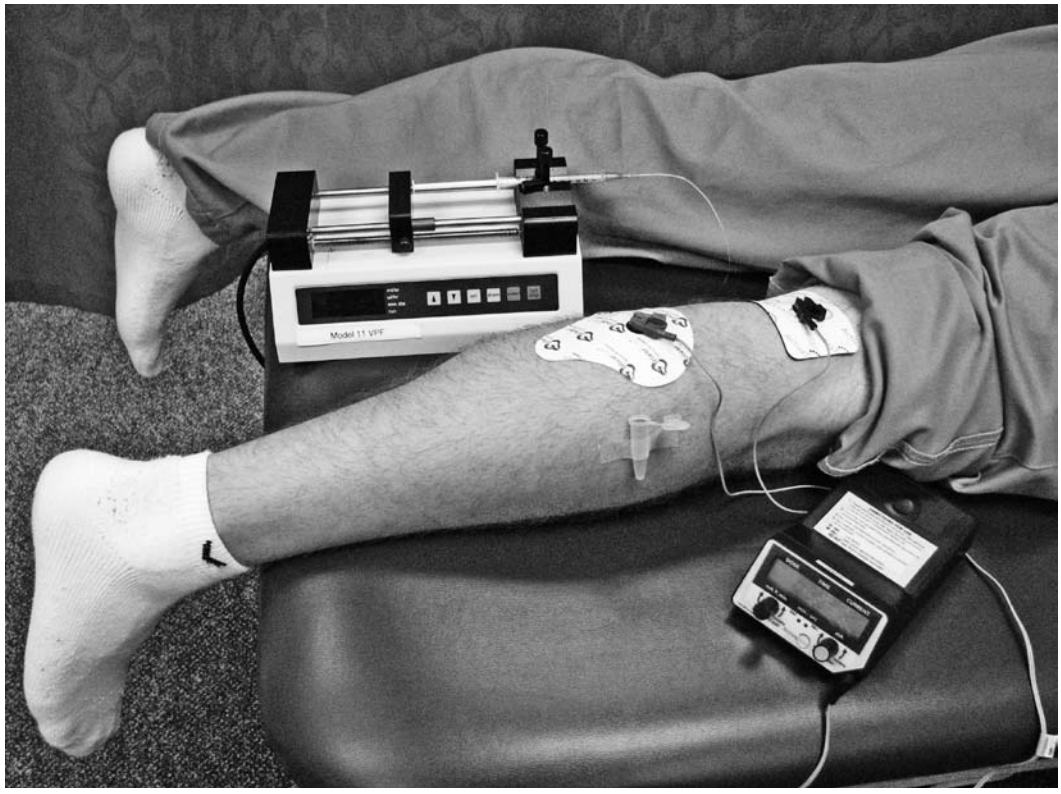

Figure 4. The experimentation setup for microdialysis and iontophoresis.

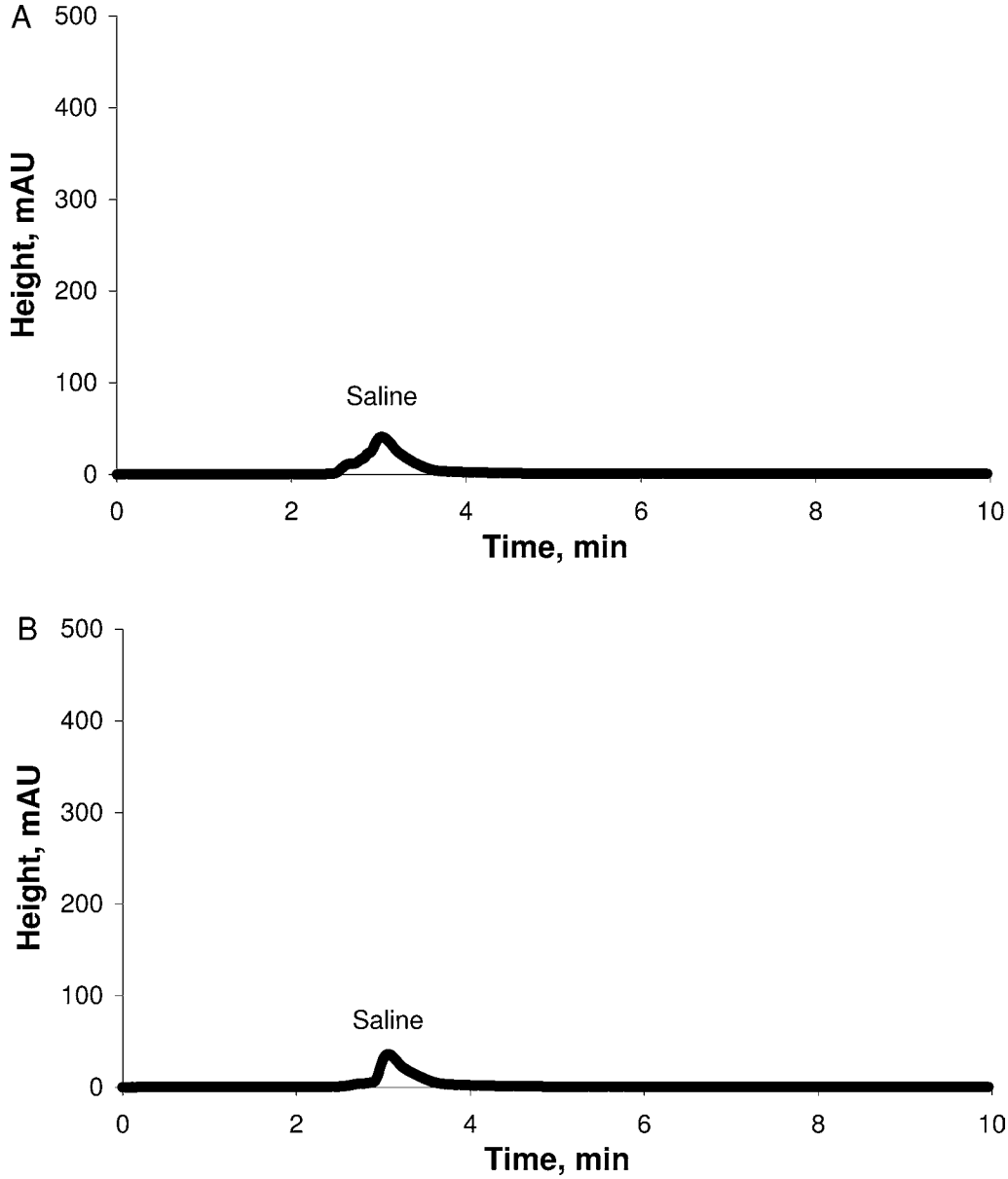

Figure 5. A, Negative high-performance liquid chromatography results at the 5-mm depth for the control leg. B, Negative high-performance liquid chromatography results at the 5-mm depth for the treatment leg.

at a current charge of 40 mA/min (Figure 4). This dose was delivered in approximately 10.5 minutes for all participants. At the end of the treatment, the iontophoresis device automatically shut off (the control leg did not receive treatment), and data collection continued for a total of 40.5 minutes (10.5 minutes during treatment and 30 minutes posttreatment). During this 60-minute interval, perfusate from the microdialysis probes in both legs was collected into separate vials for analysis. At the conclusion of the treatment, the electrodes and probes were removed from the participants. The treatment site and all portal sites were cleaned thoroughly and treated with triple antibiotic ointment. If necessary, portal sites were covered with bandages. Before dismissal, each participant was given a basic wound-care guide and our contact information. The in vivo samples were analyzed using the same RP-HPLC protocol that was used for the in vitro samples.

Statistical Analysis

When we found lidocaine present, we used paired *t* tests to compare the treatment and control legs. The α level was set a priori at .05. We used SPSS (version 17.0; SPSS Inc, Chicago, IL) for statistical analysis.

RESULTS

According to the RP-HPLC analysis, the 20 samples (10 control + 10 treatment samples) were negative for the presence of lidocaine at the 5-mm depth (Figure 5). Because lidocaine was not found in either leg, no statistical analysis to compare groups was necessary. However, the 3 treatment samples from the 3-mm depth were positive for the presence of lidocaine. Average recovery of the 1% lidocaine at the 3-mm depth was dif-

ferent between the treatment (0.5 mg/mL; range, 0.2–0.8 mg/mL) and control (0 mg/mL) legs ($t_9 = 88.655$, $P < .01$).

DISCUSSION

The use of microdialysis to measure drug-ion delivery via iontophoresis has not been discussed in the literature. Therefore, our main purpose was to verify that microdialysis was an effective tool to measure the presence of drug ions delivered via iontophoresis. We had several reasons for studying 3 instead of 10 participants at the 3-mm depth. We found the drug present in all 3 participants tested at this depth and, thus, had addressed the purpose of our experiment. In addition, because we were not comparing the 5-mm and 3-mm depths, we believed that collecting more samples was not necessary.

Difficulties With the Technique

Microdialysis generally is viewed as a practical and useful analytic technique for elucidating the concentration of key low–molecular-weight components in the ECF; however, several practical considerations need to be considered. The absolute concentration of an analyzed substance in the ECF is very difficult to determine using microdialysis. If the dialysate has a relatively high flow rate, then the analyte most likely will not fully equilibrate between perfusate (the exiting fluid from the probe) and the ECF. Therefore, HPLC, capillary electrophoresis, or other analytical measurements of the dialysate will underestimate the concentration of the analyte in the ECF.[20]

Because of this constraint, most efforts in this field have been focused on increasing the recovered analyte concentration in the dialysate. This can be done by either increasing the probe membrane surface area or reducing the flow rate, allowing more time for molecules to diffuse into the dialysate. However, increasing the probe size means that it might cause more damage to the tissue and that measurements will be less spatially precise. Reducing the flow rate reduces the temporal resolution of measurements. We considered using a larger-diameter probe; however, because such probes cost twice as much (US$200 each) as the smaller probe that we used, their use was cost prohibitive.

One way to overcome the difficulties in loss of temporal resolution with a slower flow rate is to develop better analytic techniques. Currently, the most widely used analytic technique to determine analyte concentrations in the perfusate is RP-HPLC. An ultra-slow technique can be used to generate a high recovered fraction of analyte from the ECF while still pumping the dialysate. This generates very low volumes and concentrations of dialysate, but the relative amount of analyte more closely resembles the amount of ECF. We used this technique to ensure more accurate concentrations and to minimize the loss of temporal resolution.

Limitations

Our study had some limitations, one of which was the amount and percentage of the lidocaine that was collected in the plasma solution. We were only able to measure 16% of the lidocaine added to the solution. Thus, one might ask how applicable our results are to clinical decisions. However, based on the results of the bench-top experiments, we believe that if lidocaine penetrated to the depth of the probe, the microdialysis probe would be able to collect and recover the drug even in a protein-rich environment. We are also confident that the RP-HPLC protocol used was sensitive enough to detect even small traces of lidocaine (0.005%).

Another limitation of this study may have been the absence of epinephrine in the lidocaine. Epinephrine might have helped the treatment drug reach the probe at the 5-mm depth because of its ability to cause vasoconstriction. In the future, researchers should consider using epinephrine with lidocaine when trying to detect lidocaine at levels deeper than 3 mm.

Other research in this area could involve the study of iontophoresis in other tissues, in different doses, and for longer collection times posttreatment. Future research also might involve testing topical patches, other medications, other depths, other strengths of lidocaine, and various medication compounds (eg, lidocaine with epinephrine).

Clinical Application

These results are important to the clinician who desires to numb a superficial area before needle injection or other shallow applications up to a 3-mm depth. This technique also might be applicable before cross-friction massage, myofascial release, or other unpleasant techniques used in physical medicine and rehabilitation.

CONCLUSIONS

Research regarding the effectiveness and depth of drug delivery via iontophoresis is conflicting, in part, because of less-than-ideal treatment variables, sampling, and research methods. The purpose of our study was to determine if microdialysis could recover lidocaine in the human tissues during iontophoretic delivery. Initially we attempted to do this by determining if microdialysis could detect lidocaine at 5-mm depth in the human calf. We found that microdialysis was unable to detect lidocaine at this depth. However, microdialysis detected lidocaine in all 3 participants tested at 3 mm in depth. Based on this result, we believe that microdialysis is an appropriate method to determine if lidocaine is absorbed in the human calf at a depth of 3 mm when delivered via iontophoresis.

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