

RESEARCH PROTOCOL

Predicting individual response to analgesic treatment in painful diabetic peripheral neuropathy (DPN)

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1. SYNOPSIS

Study Title	Predicting individual response to analgesic treatment with lidocaine in painful diabetic peripheral neuropathy (DPN)
Objective	To test, in a prospective, randomized, double-blind, placebo-controlled study, whether quantitative sensory measures predict the response to treatment with systemic sodium channel blocker lidocaine in patients with painful DPN.
Hypothesis	Among patients with painful DPN, those with higher mechanical pain threshold (MPT) or heat pain threshold (HPT) are more likely to respond to analgesic treatment with intravenous lidocaine.
Study Period	Planned enrollment duration: Approximately 24 months Planned study duration: 3 days per subject (1 enrollment and 2 treatment visits), with 3 week washout between the last 2 visits.
Number of Patients	35 evaluable patients with painful DPN completing the study
Study Treatment	Single intravenous 40-min infusion with 5mg/kg lidocaine or normal saline (placebo).
Study Design	Prospective, randomized, double-blind, placebo-controlled, cross-over study.
Inclusion and Exclusion Criteria	<p><u>Inclusion criteria:</u></p> <ol style="list-style-type: none"> 1. Age ≥ 18; 2. Diagnosis of Diabetes Mellitus (Fasting Plasma Glucose > 126 mg/dL and/or HbA1C $> 6.5\%$); 3. Distal symmetric pain in lower extremities with duration of more than 3 months; 4. Presence of either numbness or at least 1 sensory disturbance (increased or decreased sensitivity) in the feet. 5. Spontaneous pain with intensity of ≥ 4 on 0-10 Numerical Rating Scale (NRS). <p><u>Exclusion criteria:</u></p> <ol style="list-style-type: none"> 1. Not giving consent to participate in the study; 2. Unable to complete self-report pain questionnaire; 3. History of moderate to severe renal or liver failure; 4. History of other central or peripheral neurologic disorders; 5. History of cardiac arrhythmias; 6. Contraindication to intravenous lidocaine; 7. Pregnancy or lactation.
Measurements	<p><u>Baseline sensory testing:</u></p> <ol style="list-style-type: none"> 1. Quantitative sensory testing (QST): Thermal detection and pain thresholds, mechanical detection and pain thresholds, pressure pain thresholds, temporal summation presence, and conditioned pain modulation (CPM). 2. Spontaneous pain at baseline on 0-10 Numerical Rating Scale (NRS); 3. Assessment of pain symptoms on Neuropathic Pain Symptom Inventory (NPSI) and Brief Pain Inventory (BPI).

	<p><u>Testing for intervention response (repetitive testing):</u></p> <ol style="list-style-type: none"> 1. Spontaneous pain intensity on 0-10 NRS and 0-100 computerized visual analog scale (CoVAS); 2. Response to warm, cold, pinprick and brush stimuli on 0-10 scale (0 -not felt, 5 – normal, 10 - most intensely felt); 3. Pain descriptors on NPSI questionnaire (burning, squeezing, pins and needles, tingling, stabbing, electrical shocks) on 0-10 scale; 4. Measurement of nociception level (NoL) index with finger probe (PMD 100 device); 5. Lidocaine plasma concentrations.
<p>Statistical Methodology</p>	<p>The comparison between the analgesic effectiveness associated with each putative predictor of response (MPT and HPT) to lidocaine and to placebo will be performed by the following method: We will perform t-test in a multiple linear regression model to test the difference between slopes of predictor magnitude vs. response magnitude, namely: 1) Compare the slopes of MPT magnitude vs. lidocaine response magnitude (% reduction in pain intensity) curve, and MPT magnitude vs. placebo response magnitude (% reduction in pain intensity) curve. The same procedure will be performed for HPT predictor. The time point for primary effectiveness (pain reduction) outcome is the average of pain intensity measured at 40, 50 and 60 min after beginning of the infusion.</p>

2. STUDY PROTOCOL

2.1 Background and Significance

Diabetic peripheral neuropathy (DPN) is a neuropathic pain condition, where the pain is caused by the metabolic damage to the somatosensory nervous system, and is in fact, the leading cause of neuropathic pain worldwide. It is estimated that more than 3 million Americans suffer from DPN [1], and with projected rise in the prevalence of diabetes mellitus (DM), this number is expected to increase. The metabolic consequences of DM lead to functional and structural damage of the peripheral nerve fibers [2, 3] and long-term changes within neuronal cell bodies in the dorsal root ganglia (DRG) and in the central nervous system (spinal cord and brain). The clinical translation of the structural and functional neuronal changes in DM is yet to be fully understood, but assumed to include the characteristic features of DPN – e.g. mixed areas of increased and decreased skin sensitivity, spontaneous ongoing pain, enhanced responses to painful stimuli, and pain produced by non-painful thermal and mechanical stimuli such as cold temperatures or light touch [4-6]. The clinical effectiveness of currently available drugs to treat DPN (e.g. gabapentin, pregabalin, duloxetine) is far from satisfactory, providing significant pain relief in approximately one of every 4-5 patients [7]. However, while some DPN patients don't respond to certain treatments, others respond very well. This suggests that variability exist among the underlying pain mechanisms on the patient level, and also points to individual differences in response to analgesics. Recent literature suggests that certain methods of assessing sensory nerve function in neuropathic pain patients may provide prediction to individual analgesic response [8-10]; however, no placebo-controlled studies have been performed with the primary goal of identifying treatment response predictors in DPN (or any other neuropathic pain condition).

This approach of identifying and validating predictors of individual response to a pharmacological treatment may substantially improve treatment outcomes in diabetic patients suffering from neuropathic pain. Applying a set of sensory tests that would provide information on the likelihood of effectiveness of a specific treatment can have a significant clinically-relevant impact in reducing unnecessary side effects, burden and costs associated with “trial and error” attempts to identify efficacious individual therapy option in DPN.

One of the key mechanisms that lead to spontaneous and evoked pain characteristic in DPN is alteration in the expression of voltage-gated sodium channels both peripherally and centrally [6, 11, 12], and these channels present a pharmacological target for many of the currently used drugs (e.g. carbamazepine, oxcarbazepine, lamotrigine, amitriptyline, and mexiletine). In our recent study, we found that patients with distal symmetric polyneuropathy (primarily due to DPN) respond to systemic administration of a sodium channel blocker lidocaine better than patients with other types of neuropathic pain [13]. Moreover, certain baseline somatosensory characteristics, namely – mechanical pain threshold (MPT) and heat pain threshold (HPT) were correlated with the extent of response to lidocaine [14]. These preliminary data led us to design this prospective, double blind, placebo-controlled study with the primary objective of determining whether these somatosensory parameters predict the response to systemic lidocaine in patients with painful DPN.

Intravenous lidocaine administration is approved by the FDA for the treatment of cardiac arrhythmias, for infusion in concentrations between 4-8mg/mL.

2.2 Preliminary Data

We have previously carried out a prospective study of intravenous lidocaine in two patient populations with peripheral neuropathic pain: 1. Patients after surgical or traumatic peripheral nerve injury, 2. Patients with distal symmetric polyneuropathy such as DPN.

The study was carried out with the same administration protocol of lidocaine (5mg/kg), and has utilized a similar sensory testing protocol.

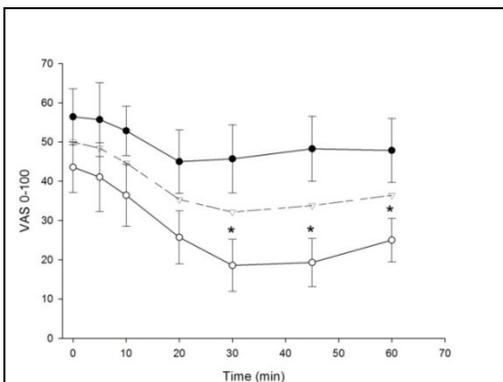


Figure 1. Intravenous lidocaine infusion resulted in significantly larger reduction in spontaneous pain intensity in polyneuropathy patients (white circles) than in peripheral nerve injury patients (black circles). White triangles represent the average group response at each point.

Patients with symmetric polyneuropathy had better analgesic response to intravenous lidocaine infusion than patients with neuropathic pain due to nerve injury (Fig. 1).

Among different sensory measures tested, the two most significant predictors of treatment response to lidocaine (in polyneuropathy patients) were MPT and HTP (Fig. 2)

The patients reported only mild adverse effects during the infusion, and no patients required treatment for adverse effects. In 6 of 14 patients the infusion was briefly paused (for 5-13 minutes) because of transient dizziness (5 patients) or blurred vision (1 patient).

This study was not placebo controlled; therefore, it is unclear whether the identified predictors of response are specific to lidocaine.

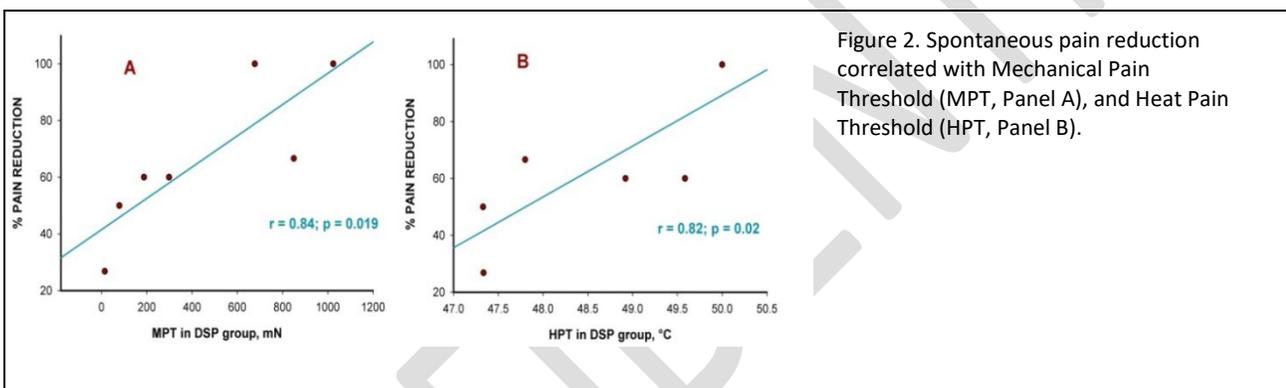


Figure 2. Spontaneous pain reduction correlated with Mechanical Pain Threshold (MPT, Panel A), and Heat Pain Threshold (HPT, Panel B).

2.3 Objective

The objective of the proposed study is to test whether quantitative sensory measures predict the response to treatment with systemic sodium channel blocker lidocaine in patients with painful DPN.

The specific aims of this study are:

1. To investigate whether the baseline mechanical pain threshold (MPT) predicts the individual response to intravenous lidocaine treatment in DPN.
2. To investigate whether the baseline heat pain threshold (HPT) predicts the individual response to intravenous lidocaine treatment in DPN.

2.4 Patient Selection

Thirty-five evaluable patients with painful DPN will be recruited from the Diabetes Center and the Pain Management Center of Washington University in St. Louis.

2.4.1 Inclusion Criteria

Inclusion criteria:

1. Age ≥ 18 ;
2. Diagnosis of Diabetes Mellitus (Fasting Plasma Glucose > 126 mg/dL and/or HbA1C $> 6.5\%$);
3. Distal symmetric pain in lower extremities with duration of more than 3 months;

4. Presence of either numbness or at least 1 sensory disturbance (increased or decreased sensitivity) in the feet.
5. Spontaneous pain with average (past week) intensity of ≥ 4 on 0-10 Numerical Rating Scale (NRS).

2.4.2 Exclusion Criteria

Subjects will not be enrolled if any of the following criteria exist:

1. Not giving consent to participate in the study;
2. Unable to complete self-report pain questionnaire;
3. History of moderate to severe renal or liver failure;
4. History of other central or peripheral neurologic disorders;
5. History of cardiac arrhythmias;
6. Contraindication to intravenous lidocaine;
7. Pregnancy or lactation.

2.5 Design and Procedures

2.5.1 Study Design

This is a prospective, randomized, double-blind, placebo-controlled, cross-over study in 35 patients with painful DPN. If our hypothesis is confirmed, then patients with higher MPT or HPT at baseline will have larger analgesic effect from lidocaine infusion.

Randomization and Blinding

Eligible patients will attend a screening visit and two intervention visits, during which intravenous lidocaine or placebo infusion will be administered in a cross-over design. At enrollment, each patient will be assigned a study number, which will match a previously prepared computer-generated list of randomization numbers to determine the sequence of interventions: lidocaine and then placebo, or *vice versa*. An unblinded investigator will be assigned to match the study number with randomized treatment sequence, and this person will prepare the study medications, which will look identical. This investigator will not be involved at any stage at patient assessment or data analysis. The participants and all other study personnel will be blinded to the treatment allocation.

Sample size

In order to identify significant differences in slopes of predictor of response magnitude vs pain reduction curve in lidocaine group and placebo group, 34 patients will be required for this cross-over trial. The calculation is based on the ability to detect difference of 14 (SD 18.21) units between correlation coefficients of the two curves, based on our pilot data [14] (the calculation was performed for 85% power, and $\alpha = 0.025$, after Bonferroni correction for two comparisons; MPT and HPT). The enrollment will continue until 35 participants have completed both phases of treatment.

2.5.2 Pre-Study Period

Potential subjects will be identified from the Pain Management Center and the Diabetes Center of Washington University in St Louis. Patients will be asked by their attending physician in the clinics for approval to provide their name and contact information to the research team. In addition, we will post flyers and recruit participants through Volunteers for Health organization. Interested subjects will contact the investigators. Once the potential subject has contacted a member of the research team, a research coordinator will provide a description of the project either by phone, email or mail, at the discretion of the potential subject. Potential subjects will be asked to undergo telephone screening by a trained research coordinator to assess preliminary eligibility for the study.

The following information will be collected:

- Age;
- History of Diabetes Mellitus;
- Presence of pain in the extremities;
- Duration of pain
- Average intensity of pain
- Presence of numbness in the feet and any changes in sensation
- History of kidney or liver failure
- Allergies to lidocaine.
- Pregnancy or lactation

Eligible patients will be invited to participate in the study, which will be conducted at Washington University Pain Management Center.

Subjects who are potential candidates for the study will be provided with written and oral description of the study procedures, benefits, and potential risks. They will be given the opportunity to ask questions regarding the study. Informed consent will take place at Washington University/Barnes-Jewish Hospital medical center campus, including the Pain and Diabetes Centers.

If agreeing to participate in the study, each subject will sign informed consent form.

Each subject who qualifies for entry into the study on the basis of inclusion/exclusion criteria and completion of informed consent will be assigned the next available patient number. This indicates enrollment in the study. In subjects with a history of or suspected renal or hepatic disorder, blood tests will be ordered for plasma creatinine, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and serum albumin. Subject with history of cardiac disease will be examined by 12-lead ECG to exclude active arrhythmias. Subjects with moderate or severe renal or hepatic failure, and subjects with active arrhythmias, will be excluded from the study at the discretion of the PI.

Patients' demographic data will be recorded; height and weight will be measured. The subject will be asked to complete the Brief Pain Inventory (BPI) AND Hospital Anxiety and Depression Scale (HADS). Subjects who drop out of the study prior to completing the study will be replaced by using the next available patient number.

2.5.3 Study Period

Subjects will be studied in the Washington University Pain Management Center. Eligible subjects will attend two treatment visits, during which intravenous lidocaine or placebo infusion will be administered in randomized order in double-blinded fashion. Subjects are instructed to refrain from eating 4 hours prior to the lidocaine infusion, but can drink clear liquids up to 2 hours prior to the procedure. The infusion start will be planned approximately at 10am; therefore subjects will be allowed to have a light snack before 6am if required for taking their DM medications (e.g. metformin). At each of these visits the subjects will undergo a quantitative sensory testing procedure to determine the MPT and HPT parameters [15]. MPT will be measured using a standardized weighted metal probes (Nervetest, MRC systems) using a modified method of levels, and HPT will be measured with Thermal Sensory Analyzer (TSA-II, Medoc, Israel) using the method of limits. In addition, other quantitative sensory measures will be obtained to better characterize the underlying nerve damage and possible pain mechanism. Namely, we will assess mechanical detection threshold (MDT) with von Frey filaments, cold and warm detection thresholds (CDT and WDT), as well as cold pain threshold (CPT) with TSA-II device, per previously published standard protocol [13, 15]. We will also apply conditioned pain modulation (CPM) protocol to assess the efficiency of descending inhibitory pain controls, as this was suggested to predict response to treatment in DPN [10], and lidocaine was shown to affect the descending inhibitory controls [16]. CPM protocol will tests whether concomitant application (conditioning) of a mildly painful stimulus on an upper extremity

(heat that elicits individually calibrated pain intensity of 30 on 0-100 NRS) reduces the painful experience from a painful (test) stimulus applied to the contralateral extremity (a thermal stimulus at temperature previously calibrated to elicit pain with intensity of 60 on 0-100 NRS). Reduction of reported pain experience to test stimulus during conditioning is a surrogate measure of efficient descending pain modulation.

At each of the intervention visits, an intravenous catheter will be inserted in the arm for drug administration, and in the opposite arm for obtaining blood samples to evaluate the plasma concentrations of lidocaine. The dose of lidocaine will be 5mg/kg ideal body weight (IBW), calculated by B. J. Devine Formula:

$$IBW = 50.0 + 2.3 \text{ kg per inch over 5 feet} \quad (\text{man})$$

$$IBW = 45.5 + 2.3 \text{ kg per inch over 5 feet} \quad (\text{woman})$$

Lidocaine will be infused during 40 minutes. The unblinded investigator will prepare 100mL normal saline infusion bags either with lidocaine or the same volume of normal saline (placebo). For lidocaine infusion, saline will be first drawn from the infusion bag in a volume equal to lidocaine volume to be added to the infusion, to keep the same total volume of the infusion bag. The total lidocaine dose will be limited to 500mg for safety reasons. The subjects will have continuous 3-lead ECG monitoring for 90 minutes.

Prior to infusion, we will assess:

1. Spontaneous pain intensity on 0-10 NRS.
2. NPSI pain descriptors burning, squeezing, pins and needles, tingling, stabbing, electrical shocks) on 0-10 scale
3. Evoked response (0-10 NRS) to cold (20°C, Rolltemp, Somedic), warm (40°C, Rolltemp, Somedic), brush (Senselab brush-05, Somedic) and pinprick (256mN metal probe, MRC Systems) stimuli applied to a dorsal foot and a non-painful control site (ipsilateral shoulder).

Tabular description of the study assessments is available in Table 1.

Table 1. Data assessment schedule

Required Assessments	Baseline visit	Treatment Visit 1	Washout			Treatment visit 2	Week 1b	Week 2b	Week 3b	End of Study communication
			Week 1a	Week 2a	Week 3a					
Informed consent	✓									
Inclusion/exclusion criteria	✓									
Randomization	✓									
Demographic data collection	✓									
BPI questionnaire	✓									
HADS questionnaire	✓									
Sensory Mapping	✓									
Assessment of sensitivity to cold, warm, brus and pinprick		✓				✓				
NoL measurement		✓				✓				
NPSI questionnaire		✓				✓				
QST		✓				✓				
CPM		✓				✓				
Blood sampling for PK		✓				✓				
Pain intensity 0-10 NRS		✓	daily	daily	daily	✓	daily	daily	daily	✓
Adverse Event assessment		✓				✓				✓
Blinding questionnaire										✓

With the beginning of the infusion, pain and the above mechanical and thermal evoked responses will be assessed every 10 minutes for 60 minutes, and then spontaneous pain will be assessed every 15 minutes for the next hour (total 120 min from the beginning of infusion). NPSI will be assessed at 60 min post-infusion.

In addition, skin temperature and blood flow will be assessed prior to and intermittently during the study drug infusion by an epidermal patch that rests on the skin surface. The thermal sensors, referred to as epidermal transient plane source (ETPS) sensors, utilize the well-established transient plane source method toward the identification of the thermal characteristics of skin. The ETPS sensor capabilities have been thoroughly studied [17], and the device concepts have been employed successfully in clinical studies related to blood flow [18], dermatological health [19], thermal transport properties of skin [20, 21], and wounds [22]. The measures that the ETPS sensors—cutaneous blood flow and temperature—are variables that have clear associations with inflammation and nociception, and can be used in this study as additional physiologic descriptors of the patient's disease and pain state.

Blood samples (4mL tubes) for determining lidocaine concentration will be obtained at baseline, and at 20, 45, 60, 90, and 120 minutes after beginning of the infusion. The samples will be put on ice, centrifuged for 10 min at 5000 rpm, plasma will be transferred to 1.5mL vials and stored at -80°C until analysis by HPLC-MS.

After discharge, average daily spontaneous pain intensity on 0-10 NRS will be recorded in a diary (q pm) for 3 weeks after each infusion. The infusions will be administered 3 weeks apart, as some studies have reported analgesic effect of a single lidocaine infusion lasting for up to 14 days.

ECG and oxygen saturation by pulse oximetry will be monitored throughout the infusion and for approx. 60 min after the end of infusion. Meals and oral fluids will be provided 1 hour after the end of lidocaine infusion. Subjects will be discharged from the unit after a minimum stay of 90 minutes after the end of infusion, and if no neurological or cardiac adverse effects are present, or when any such effects have completely resolved. In case of continuing adverse effects, the subjects will be monitored by the study staff until resolution of these effects as determined by the PI. At the end of the session the IV will be removed and subjects may go home. Subjects are not allowed to drive or operate machinery on the day of the study session.

Intravenous lidocaine infusion may cause dizziness and drowsiness; therefore, there is a risk of falling if the patient attempts to walk without assistance soon after the end of infusion. Subjects will be monitored to ensure they do not stand up or walk without assistance for up to 90 min after the end of infusion. The patients will be required to have a family member escort them home, and transportation will be provided if necessary.

2.5.4 Minimization of Bias

There will be no specific sex, ethnic or racial background for enrollment. Placebo arm is introduced to increase the ability to differentiate between the pharmacologic effects of lidocaine and other possible effects. The study is designed as cross-over in order to minimize inter-subject variability. The investigators and the subjects will be blinded to treatment allocation sequence. Plasma lidocaine concentration is not affected by subjects' or investigators' knowledge of study aims and hypothesis.

2.5.5 Observations and Measurements

2.5.5.1 Primary Outcome Measures

Primary outcome:

Reduction in spontaneous pain intensity on 0-10 NRS in the feet at 50 minutes after beginning the intravenous lidocaine infusion. This endpoint is calculated as the average of pain intensities measured at 40, 50 and 60 min after beginning of the infusion.

2.5.5.2 Secondary Outcome Measures

Secondary outcomes:

- 1). Change in the intensity (0-10 scale) of evoked mechanical and thermal sensation: cold, warm, brush and pinprick.
- 2). Change in the NPSI descriptors of pain (burning, squeezing, pins and needles, tingling, stabbing, electrical shocks) on 0-10 scale at 60 min post-infusion.
- 3). Multiple regression analysis to investigate how the combination of various sensory phenotypes assessed in this study (i.e. MPT, HPT, MDT, WDT, CDT, CPT, and CPM) and lidocaine pharmacokinetics (area under the concentration-time curve, (AUC) and maximal plasma concentration (Cmax)) correlate with treatment response to lidocaine.

2.5.6 QST protocol

Quantitative sensory testing will be performed on the dorsal mid-foot. If asymmetry in pain intensity exists between extremities, QST will be performed in the more painful foot; otherwise the foot will be chosen randomly. The ipsilateral shoulder will serve as control area.

A description of the QST procedures follows:

Thermal detection and thermal pain thresholds

Equipment: The Thermal Sensory Analyzer (TSA-II platform - Medoc, Ramat Yishai, Israel) will be used to determine thermal detection and pain thresholds. This equipment is used globally for functional assessment of pain and temperature-conducting nerve fibers (C and A-delta fibers).

Method and Background: Using the thermal sensory analyzer, cold and warm detection thresholds (CDT and WDT, respectively), as well as cold and heat pain thresholds (CPT and HPT, respectively) will be determined [23, 24]. The thermode with contact area of 9.0 cm² is applied to the tested site, and all thresholds are determined by continuous ramping of temperature from 32°C baseline temperature by 1°C/s until the subject presses the ‘stop’ button. Cut-off temperatures are 0°C and 50°C, to minimize thermal damage to the skin. The baseline temperature to which the thermode returns before each test is 32°C. The average threshold is calculated from three measurements in each area.

Determination of mechanical detection threshold (MDT)

Equipment: A set of standardised von Frey filaments (0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256mN). The contact area of the hairs with the skin is of uniform size (<1 mm²) and texture.

Methods and Background: Standardised von Frey filaments will be used in a modified “method of limits” manner using 3 series of increasing and decreasing stimulus intensities to determine the geometric average as the tactile detection threshold of the affected and unaffected skin areas [25].

Von Frey filaments of different stimulus intensities are used to determine the tactile detection thresholds. A filament eliciting 16mN force* is applied first, followed by filaments of consecutively lower intensity until the patient cannot detect the stimulus being applied. This respective force represents the first threshold value. The order in which the stimuli are applied is then reversed and stimuli of consecutively greater intensity are applied until sensation is detected (this intensity becomes the second value). Again filaments with decreasing intensity are applied until in total 3 upper and lower values of detection are fulfilled from which the mechanical detection threshold can be determined.

* In case the first von Frey filament with an intensity of 16mN is not detected, the next highest intensity filament which can be detected must be used as a starting intensity. However, the relevant force of this stimulus is not documented. Filaments with consecutively lower intensity are applied until the patient

cannot detect the stimulus being applied. The procedure is followed as above; until in total 3 upper and lower values of detection are fulfilled from which the mechanical detection threshold can be determined.

Determination of mechanical pain thresholds (MPT)

Equipment: A set of standardized weighted metal probes (Nervetest, MRC systems) exerting pressure of 8, 16, 32, 64, 128, 256 and 512 mN.

Methods and Background:

The standardized metal probes will be used in a modified method of levels manner, 3 series of increasing stimulus intensities to detect the mechanical pain threshold. Beginning with an applied force of 8mN, stimuli increase in intensity until the sensation induced by increased pressure can be described as 'painful'. The corresponding force is used to represent the first MPT value. The procedure is then repeated a total of 3 times and until a total of 3 values are obtained, from which the mean MPT is determined.

Determination of wind-up ratio (WUR)

Equipment: A standardized weighted metal probes (Nervetest, MRC systems) exerting pressure of 256mN.

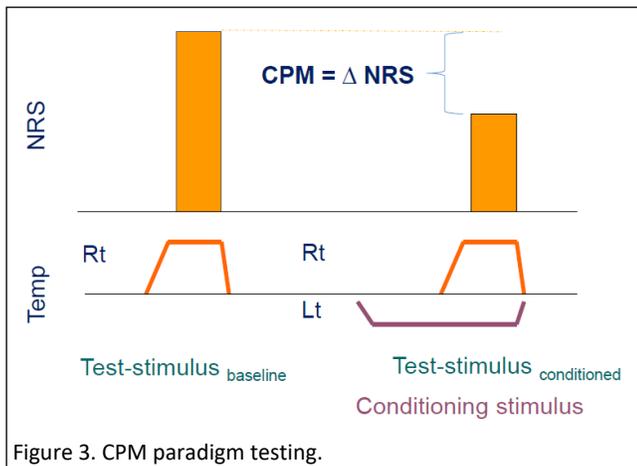
Methods and Background: In this test a pinprick (256mN) is first applied singularly. After that a series of 10 identical pinprick stimuli are applied with a frequency of 1 s^{-1} within an area of 1 cm^2 . Immediately following the single stimulus and series of stimuli, an evaluation of the sensation must be provided according to NRS (0-10, '0': 'no pain', '10': 'worst pain imaginable'). A ratio is calculated using these values. This procedure will be repeated twice. A geometric average of the 'wind-up' is calculated from the two ratios [26, 27].

Determination of conditioned pain modulation (CPM) efficiency

Equipment: The Q-Sense Thermal Analyzer (Medoc, Ramat Yishai, Israel) will be used for CPM paradigm testing.

Methods and Background

The Q-Sense works on the same principle as TSA-II, which is used for determining the thermal thresholds. However, Q-Sense is equipped with two 3x3cm Peltier thermodes – one used as conditioning stimulus, and the other used as test stimulus. The intensity of the conditioning stimulus will be determined individually by temperature that elicits pain intensity of 30 on 0-100 NRS. The intensity of test stimulus will also be determined individually, at the temperature that elicits pain intensity of 60 on 0-100 NRS. The conditioning stimulus will be applied at left shoulder, while the test stimulus will be applied at the right shoulder. The length of the conditioning stimulus is 60 seconds, during the last 30 seconds of which the pain stimulus is applied. The stimuli are applied according to the diagram in Fig.3 below – and the difference between the intensity of pain stimulus without conditioning and between the intensity of pain stimulus with concomitant conditioning is the CPM magnitude. $\text{CPM} > 0$ implies efficient descending pain modulation.



2.5.7 Statistical Methods

The comparison between the analgesic effectiveness associated with each putative predictor of response (MPT and HPT) to lidocaine and to placebo will be performed by the following method: We will perform t-test in a multiple linear regression model to test the difference between slopes of predictor magnitude vs. response magnitude, namely: 1) Compare the slopes of MPT magnitude vs. lidocaine response magnitude (% reduction in pain intensity) curve, and MPT magnitude vs. placebo response magnitude (% reduction in pain intensity) curve. The same procedure will be performed for HPT predictor.

The time point for primary effectiveness (pain reduction) outcome is 50 min after beginning of the infusion, based on our preliminary data. This will be calculated from the average of pain intensities at 40, 50 and 60 min from the beginning of the infusion.

The time point for secondary outcome of change in NPSI pain descriptors is 45 min after beginning of the infusion.

In exploratory phase, multiple regression analysis will be performed to investigate how the combination of various sensory phenotypes assessed in this study (i.e. MPT, HPT, WDT, MDT, CDT, CPT, and CPM) and lidocaine pharmacokinetics (area under the concentration-time curve, (AUC) and maximal plasma concentration (Cmax)) correlate with treatment response to lidocaine.

2.5.8 Sample Size

In order to identify significant differences in slopes of predictor of response magnitude vs pain reduction curve in lidocaine group and placebo group, 34 patients will be required for this cross-over trial. The calculation is based on the ability to detect difference of 14 (SD 18.21) units between correlation coefficients of the two curves, based on our pilot data [14] (the calculation was performed for 85% power, and $\alpha = 0.025$, after Bonferroni correction for two comparisons; MPT and HPT). The enrollment will continue until 35 participants have completed both phases of treatment.

2.5.9 Clinical Procedures and Laboratory Tests

Due to the risk of decreased clearance of lidocaine in participants with moderate to severe renal or hepatic insufficiency, active surveillance to prevent drug accumulation will be performed. Plasma creatinine and liver transaminases will be determined before the study procedure in subjects with history of or suspected renal or hepatic disorders. If renal insufficiency ($SCr >1.5$ mg/dL or creatinine clearance < 50 mL/min/1.73m² [by Cockcroft-Gault formula]) or hepatic insufficiency (ALT or AST more the twice the

upper normal limit, or serum albumin below 3.5 g/dL) is present, the subject will be withdrawn from the study.

Due to increased risk of cardiac arrhythmias with lidocaine, patients with history of arrhythmias will be excluded. To increase safety, all patients with history of cardiac disease will be examined by 12-lead ECG to exclude active arrhythmias.

The patients will continue their chronic medications; including chronic pain medications, provided the dose have been stable for at least 3 weeks. Any PRN analgesics will not be allowed for 24 hours prior to each of the study visits.

3.0 Management of Intercurrent Events

3.1 Adverse Experiences

The investigator will closely monitor subjects for evidence of systemic lidocaine adverse events. All adverse events will be reported and followed until satisfactory resolution. The description of the adverse experience will include the time of onset, duration, severity, etiology, relationship to the study drug (none, unlikely, possible, probable, highly probable), and any treatment required.

In case of mild tachy- or brady- arrhythmias, or oxygen desaturation, the infusion will be paused, and attempted to resume 5 minutes after resolution of the adverse effect. The resumed infusion rate may be reduced from 7.5mg/kg/h to 5mg/kg/h, at the discretion of the PI. In any case of moderate to severe tachy- or brady- arrhythmias, or oxygen desaturation, the infusion will be stopped, and patient withdrawn from the study. In any case of serious side effects, the study team will follow the American Society of Regional Anesthesi (ASRA) guidelines on systemic local anesthetic toxicity [28]. Resuscitation kit and Intralipid[®] infusion for possible local anesthetic systemic adverse effect management will be readily available.

3.2 Premature Discontinuation

If a subject withdraws from the study, the subject will be replaced in order to provide the required number of evaluable subjects. Subjects will be withdrawn if the investigator decides that discontinuation is in the best interest of the subject, or the subject requests withdrawal from the study.

3.3 Potential Risks

3.3.1 Potential risks from lidocaine

Local anesthetic systemic effects associated with high plasma concentration may cause cardiac arrhythmias or neurologic adverse effects such as dizziness or drowsiness. Muscular twitching, convulsions and unconsciousness have been reported with inadvertent high dose intravascular boluses. Patients may experience blurred vision, dry mouth or perioral numbness. However, we have used this exact protocol in studies previously, and no patient had to discontinue treatment because of adverse effects. Moreover, in this study we will base the dosing on ideal body weight instead of actual weight, which will result in lower lidocaine doses, and eventually reduced risk of adverse effects. Intravenous lidocaine is FDA-approved for the treatment of cardiac arrhythmias, used by infusion in concentrations between 4-8mg/mL. In this study, the final concentration of infused lidocaine is expected to be between 4-6mg/mL, based on subjects' IBW, which falls within the approved concentration and dosage. We have limited the allowed dose to 500mg lidocaine to prevent serious systemic adverse effects. The serious systemic adverse effects of local anesthetics have typically occurred from inadvertent intravascular bolus injections of solution intended for regional anesthesia. Adverse effects such as muscle twitching, unconsciousness and convulsions have been usually reported following plasma lidocaine concentrations of >12 mcg/mL. In this study, lidocaine is administered by slow IV infusion, and the resulting peak plasma concentrations are not expected to exceed 3-4mcg/mL, per previous studies. However, in case of

any serious side effects, the study team will follow ASRA guidelines on systemic local anesthetic toxicity [28]. No psychological risks to subjects are envisioned.

3.3.2 Potential risks from thermal testing

Risk of injury related to thermal pain testing is minimal. Thermal testing is widely used and safe. While thermal testing does produce pain, risks to the individual are minimal, because 1) the pain is transient in nature and generally subsides immediately after the procedure; 2) subjects are instructed that they may stop any procedure at any time with no adverse consequences; and 3) the level of pain experienced by subjects is below their tolerance level. With thermal stimulation there is a very slight risk of a burn, but this is minimized by the following: 1) positive lockout of stimulus parameters above 52°C; and 2) the stimulator has built in a shut-down system to prevent the delivery of prolonged or high intensity stimuli. Both TSA-II and Q-Sense have FDA 501(k) clearance (K922052).

3.3.3 Other Potential Risks

Intravenous catheter placement can cause a bruise. The amount of blood drawn is approx. 30cc and will not constitute a risk to subjects since this amount is well below the recommended limits for this population. No psychological risks to subjects are envisioned. Subjects may experience a loss of confidentiality. Investigators will keep subjects' participation confidential to the extent permitted by law. However, it is possible that others may become aware of subjects' participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies subjects.

3.4 Procedures to Minimize Potential Risks

Studies are conducted in the Washington University Clinical Research Center under the supervision of the PI and the co-investigator. The PI is trained and experienced in performing research in human subjects, and in monitoring local anesthetic adverse effects. The co-investigator is board certified anesthesiologist with extensive experience in local anesthetic administration and monitoring.

Subjects are also continuously monitored by trained (RN) nursing personnel. Subjects will be continuously monitored by electrocardiogram for any potential cardiac abnormalities associated with local anesthetic administration. Full patient monitoring and resuscitation capabilities are immediately available. Subjects are kept under observation in the Pain Management Center. They are instructed not to walk without support for 90 minutes after the end of lidocaine infusion, and not to drive for the rest of the day.

Inclusion and exclusion criteria, fasting requirements, monitoring, and the clinical protocol are designed to ensure that risks are absolutely minimal. Subjects are informed that participation is voluntary and they may refuse to participate and may withdraw from the study at any time without penalty. A pregnancy test will be performed on women of childbearing potential and subjects excluded if pregnant. Subjects will be told that in the event of a physical injury as the direct result of study procedures, they will be cared for by a member of the investigating team at no cost, within the limits of the Washington University compensation plan.

Subjects will be instructed prior to each infusion session, that at any point they experience bothersome side effects, they can ask the investigator to pause the study drug infusion. After holding the infusion for 5 minutes, the investigator will assess the subject's side effects. If the adverse effect has disappeared/improved, and the subject is willing to resume the infusion, it will be continued, with the coordinator documenting the length of infusion hold (minutes). This can be repeated as many times as the subjects feels necessary during the infusion. The subject can ask to stop the infusion session voluntarily at any time and withdraw from the session or from the entire study.

In case

With regard to confidentiality; 1) all subjects will be assigned a study ID number, 2) Samples will be kept confidentially. They will be coded, with a key to the code linking code numbers to names kept at a separate location, under lock and key. 3) The link to identifiers will be destroyed at the end of the study. 4) Data will be stored under lock and key (office, file cabinet) and only the investigators and research team will have access. If data are published, there will be no link to identifiers. Study data will not be revealed to any organization, individuals other than the subjects, or the subjects themselves. 5) Study data will not be entered in subjects' medical records.

3.5 Data and Safety Monitoring Plan

The specific monitoring plan for this investigation is commensurate with the risks and the size and complexity of the investigations planned. The potential risks are attributable to the local anesthetic lidocaine and normal saline (placebo) infusion. Based on these considerations, the monitoring plan involves engaging a colleague from the Department of Anesthesiology not involved in the study to serve in a monitoring capacity. Based on the small size and relatively low risks nature of the protocol, only a third person (the colleague), rather than a full Data Safety Monitoring Board will be used. The colleague will be an anesthesiologist knowledgeable in the risks associated with nerve blocks and local anesthetic administration. This individual will review the annual summary of adverse events. In addition, this colleague will review all reports of a Serious Adverse Event, or an Unexpected Adverse Event.

4. HUMAN SUBJECTS RESEARCH

4.1 Protection of Human Subjects

The study will be conducted under appropriate Washington University Institutional Review Board protocols and consent forms approvals. The study will be conducted under the supervision of the PI, a GCP-certified pharmacist with several years of experience in the conduct of human studies, and co-PI, a board-certified Anesthesiologist with extensive clinical and human research experience.

4.2 Sources of Materials

Subjects will be recruited from Washington University Diabetes Center and Pain Management Center.

Data on comorbidities and concomitant medication use are provided by subjects. Specimens include blood obtained exclusively for determining lidocaine plasma concentration. Other data including baseline quantitative sensory testing are obtained exclusively for research purposes.

4.3 Recruitment and Informed Consent

Participants will be recruited primarily through Washington University Diabetes Center and Pain Management Center, referred by the corresponding physicians. In addition, we will post flyers and recruit participants through Volunteers for Health organization. Interested subjects will contact the investigators. Subjects will be given verbal (initially) and then written descriptions of the study aims, procedures, risks, and benefits, and will be required to give written informed consent. A member of the investigative team provides all study descriptions, informed consent, and answers all questions. Placebo infusion is a part of the study, and the subjects will be informed that they will be receiving placebo at one of their study visits. Subjects are informed verbally and in writing that participation is voluntary and they may refuse to participate and may withdraw from the study at any time without penalty.

4.4 Potential Benefits of the Proposed Research to the Subjects and Others

The potential benefit to the study subjects is a temporary (1-2 days) relief of their chronic pain. Some studies have reported long-term (up to 2 weeks) pain relief with single administration of intravenous

lidocaine, but we do not know if this long-term effect is likely in this group of patients. Potential understanding of which patients are more likely to respond to analgesics with sodium-channel blocking properties may lead to improved patient outcomes from which the study subjects and other subjects suffering from painful DPN may potentially benefit in the future.

The society may benefit from a new approach of optimizing treatment of neuropathic pain.

4.5 Inclusion of Women

Studies actively encourage the participation of women in the research. As a matter of operational policy, our studies routinely and deliberately attempt to include equivalent numbers of women and men. However, the nature of the current study precludes enrollment of a set number of female or male patients since the main criteria for inclusion is painful diabetic neuropathy. Women of childbearing potential are not excluded from our research protocols.

4.6 Inclusion of Minorities

All of our studies actively encourage the participation of minorities in the research. Our minority recruiting typically matches the demographic composition of the Washington University community from which subjects will be recruited (78% white, 21% Black, <1 % Hispanic).

4.7 Inclusion of Children

Children <18 yr will not be studied in this investigation, because painful diabetic peripheral neuropathy is uncommon in this population. Including children may expose them to an unnecessary risk without the benefit of generalizability of the results.

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