RESEARCH PROTOCOL

Administration of ondansetron with P-glycoprotein inhibitor tariquidar in patients with neuropathic pain

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Version Date: September 8, 2020
Revised: October 29, 2020
April 21,, 2022

Short Title: Ondansetron-Tariquidar combination in patients with neuropathic pain

1. SYNOPSIS

Study Title	Administration of ondansetron with P-glycoprotein inhibitor tariquidar in patients with neuropathic pain
Objective	To determine the pharmacokinetics and tolerability of co-administration of 5-HT_3R antagonist ondansetron with a P-glycoprotein inhibitor tariquidar, in patients with neuropathic pain.
Hypothesis	Co-administration with tariquidar will increase the cerebrospinal fluid (CSF) to plasma ratio of ondansetron after intravenous administration. Co-administration of a single dose 16mg ondansetron with 4mg/kg tariquidar will be tolerable in patients with neuropathic pain
Study Period	Planned enrollment duration: Approximately 24 months Planned study duration: Approximately 30 months
Number of Subjects	28 evaluable patients with peripheral neuropathic pain
Study Drug	Two infusions of 16mg intravenous ondansetron co-administered with intravenous 4mg/kg tariquidar or placebo (D5W), 3 weeks apart. If intolerable, the dose of ondansetron will be reduced to 12mg or 8mg in a stepwise fashion (see section 3.2).
Study Design	Prospective, randomized, double-blind, placebo controlled, cross-over proof of concept study.
Inclusion and Exclusion Criteria	Inclusion criteria: 1. Age 18-70; 2. Documented diagnosis of neuropathic pain due to damage or disease affecting the peripheral nervous system; 3. At least Probable neuropathic pain grading[1]; 4. Pain duration >3 months; 5. Average pain intensity ≥4 on 0-10 numerical rating scale (NRS). Exclusion criteria: 1. Current pregnancy or lactation; 2. Moderate-severe kidney or liver dysfunction; 3. Active cardiac arrhythmias (non-sinus rhythm), Long QT syndrome, or QTc interval >450msec; 4. Congestive heart failure 5. Abnormal troponin values at screening visit; 6. Current treatment with MAO inhibitors, mirtazapine, SSRI or SNRI antidepressants, except duloxetine up to 60mg/day, escitalopram up to 10mg/day, or citalopram up to 20mg/day; 7. Current treatment with tapentadol, tramadol, or fentanyl; 8. Current treatment with P-glycoprotein substrate drugs with narrow therapeutic window, e.g. digoxin; 9. Current treatment with tricyclic antidepressant medications (e.g. amitriptyline, desipramine, imipramine) at a dose >25mg/day; 10. Ongoing use of any of the following medications with known effects on Pgp function: carbamazepine, phenytoin, phenobarbital, cyclosporine, clarithromycin, erythromycin, ritonavir, verapamil, rifampicin, St. John's wort; 11. Current treatment with QT-prolonging drugs, and drugs known to have a significant interaction with ondansetron or other P-glycoprotein substrates (see section 2.3.3.);

Measurements	1. Venous blood sampling for pharmacokinetics: At 0, 15, 30, 60, 90, 120, 180, and 240 minutes from the beginning of ondansetron infusion. 2. A CSF sample will be obtained via lumbar puncture between 30 and 180 minutes from the beginning of ondansetron infusion, for the assessment of Ondansetron concentration in the CSF using population PK approach. 3. Vital signs (pulse oximetry, blood pressure): before infusion and 30, 60, 120, 180 and 240 minutes after end of ondansetron infusion. 4. 12-lead ECG: At baseline and prior to discharge. 5. Troponin: prior to discharge. 6. Intensity of spontaneous pain (on 0-10 NRS): At baseline, and after ondansetron administration; every 10 minutes for 60 minutes, every 15 minutes thereafter for a total of 120 minutes, every 30 minutes until 240 minutes, hourly on the study day until bedtime, and daily for 3 weeks thereafter. 7. Neuropathic Pain Symptom Inventory (NPSI) pain descriptors: Prior to and 70 minutes after ondansetron administration.
Statistical Methodology	<u>Primary Outcome:</u> The primary objectives are to evaluate the pharmacokinetics and the tolerability of ondansetron and tariquidar single dose iv co-administration (in comparison to ondansetron administration alone) in patients with neuropathic pain. <u>Secondary outcomes</u>: 1. Change in spontaneous pain intensity (measured on 0-10 NRS) from baseline to 60-120 minutes after ondansetron IV infusion, compared between two sessions with and without tariquidar. The pain severity values obtained at 60, 90, and 120 minutes will be averaged to derive the outcome measure. The values will be compared via paired t-test (or Wilcoxon signed-rank test, if data not normally distributed). 2. Changes in NPSI total score and sub-scores (burning pain, paroxysmal pain, paresthesia/dysesthesia score) will be compared between treatment sessions; 3. The association between baseline Conditioned Pain Modulation (CPM) magnitude (ΔCPM) and the % pain reduction from baseline will be determined by bivariate regression. A significant association between the two measures will be indicative of differential analgesic response as a function of descending pain modulation; 4. Exploratory analyses will be performed to determine if variables such as sex, weight, baseline wind-up ratio (WUR), peak ondansetron plasma concentration, and P-glycoprotein genotype affect the difference in pain reduction between the two study sessions.

2. STUDY PROTOCOL

2.1 Background and Significance

Neuropathic pain (NeuP) affects more than 16 million Americans[2] and results in considerable impairment in quality of life.[3] Five of every six patients with NeuP do not achieve adequate pain relief with currently approved medications;[4] moreover, efficacious drugs such as opioids and tricyclic antidepressants have major safety concerns. Therefore, new therapeutic approaches are urgently needed to reduce the burden of NeuP.

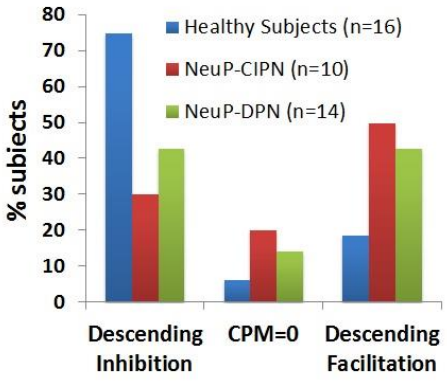
Serotonergic 5-HT_3 receptors (5-HT_3R) in the central nervous system (CNS) have been identified as a promising pharmacological target for neuropathic pain.[5,6] Normally, descending modulation from the brainstem inhibits the sensitization of spinal cord dorsal horn neurons through serotonin activity.[7] However, after peripheral nerve damage, neurons in the dorsal horn demonstrate overexpression of 5-HT_3R, the only (excitatory) ion channels among serotonin receptors.[6,8] As a result, the character of descending serotonergic modulation in NeuP can shift from inhibitory to excitatory (facilitatory).[9,10] This finding is supported by studies demonstrating that local intrathecal delivery of 5-HT_3R antagonists such as ondansetron alleviates mechanical and thermal hypersensitivity in animal models of nerve injury.[5,11-13] In contrast, these analgesic effects are not consistently observed with systemic administration of these drugs in either preclinical pain models[14,15] or in analgesic clinical trials.[16,17] This discrepancy can be explained by the fact that currently available 5-HT_3R antagonists are substrates of efflux transporters such as the P-glycoprotein (Pgp), located at the capillaries of blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB).[18,19] Our preliminary data and others[20] suggest that this efflux limits the CNS exposure to systemic 5-HT_3R antagonists, preventing neuropathic pain relief. Bypassing Pgp-mediated efflux is expected to increase the concentrations of these drugs at their CNS sites of action and thus provide adequate relief in neuropathic pain.

In addition to CNS penetration, the choice of appropriate pain condition and patient somatosensory characteristics are important for preventing a "*translational mismatch*" between preclinical and clinical study design.[21] While 5-HT_3R overexpression in rodent nerve injury models contributes to the descending facilitation,[11,22] none of the previous clinical studies with 5-HT_3R antagonists have attempted to select patients who would exhibit a phenotype suggesting descending facilitation, where the intervention is expected to work. Moreover, in one study that undertook the effort of quantifying descending modulation, most patients exhibited efficient inhibition (and not facilitation).[23] Interestingly, tropisetron was efficacious in fibromyalgia,[24,25] a condition in which most patients tend to exhibit descending facilitation.[26,27] Determining descending pain modulation phenotype is promising for predicting responses to certain analgesics,[28,29] and recent recommendations for such sensory phenotyping in chronic pain studies have been published.[30]

2.2 Preliminary Data

Our preliminary data on conditioned pain modulation (CPM), a surrogate measure of descending inhibition vs. facilitation (**Fig. 1**) demonstrate that while in healthy adults less than 20% exhibit descending facilitation, 40-50% of patients with peripheral NeuP exhibit various degrees of descending facilitation. We expect to have sufficient balance between descending inhibition and facilitation among enrolled NeuP patients, to determine the association between this phenotype and pain reduction. In a recent pharmacokinetic study of ondansetron disposition in the CSF, a 7-fold difference was observed between plasma and CSF concentrations of ondansetron after a single 16-mg infusion.[31]

Fig 1. Conditioned pain modulation (CPM) in NeuP and healthy controls.



DPN- diabetic peripheral neuropathy; **CIPN**- chemotherapy-induced peripheral neuropathy;

We have also performed a series of experiments in rats to determine the distribution properties of ondansetron (in plasma, heart, and brain) in the setting of P-glycoprotein (Pgp) genetic knockout (KO) or Pgp inhibition with tariquidar (Figure 2). Plasma concentrations (2A) of ondansetron were not different between wild type (WT) rats, Pgp KO rats, or after pre-administration of 15mg/kg tariquidar in

wild-type rats. Heart concentrations (2B) of ondansetron in Pgp KO rats compared with WT rats were not different at 30 and 60-min time points, and were lower at 15- and 120-min time points. In rats pre-treated with tariquidar (only 1-h time point available), the heart concentrations of ondansetron were not different from wild-type or Pgp knockout. Brain concentrations (2C) of ondansetron were higher in Pgp KO rats and in tariquidar-treated rats, compared with ondansetron administered to WT rats. There was no difference in brain concentrations between Pgp KO rats and rats pre-treated with tariquidar.

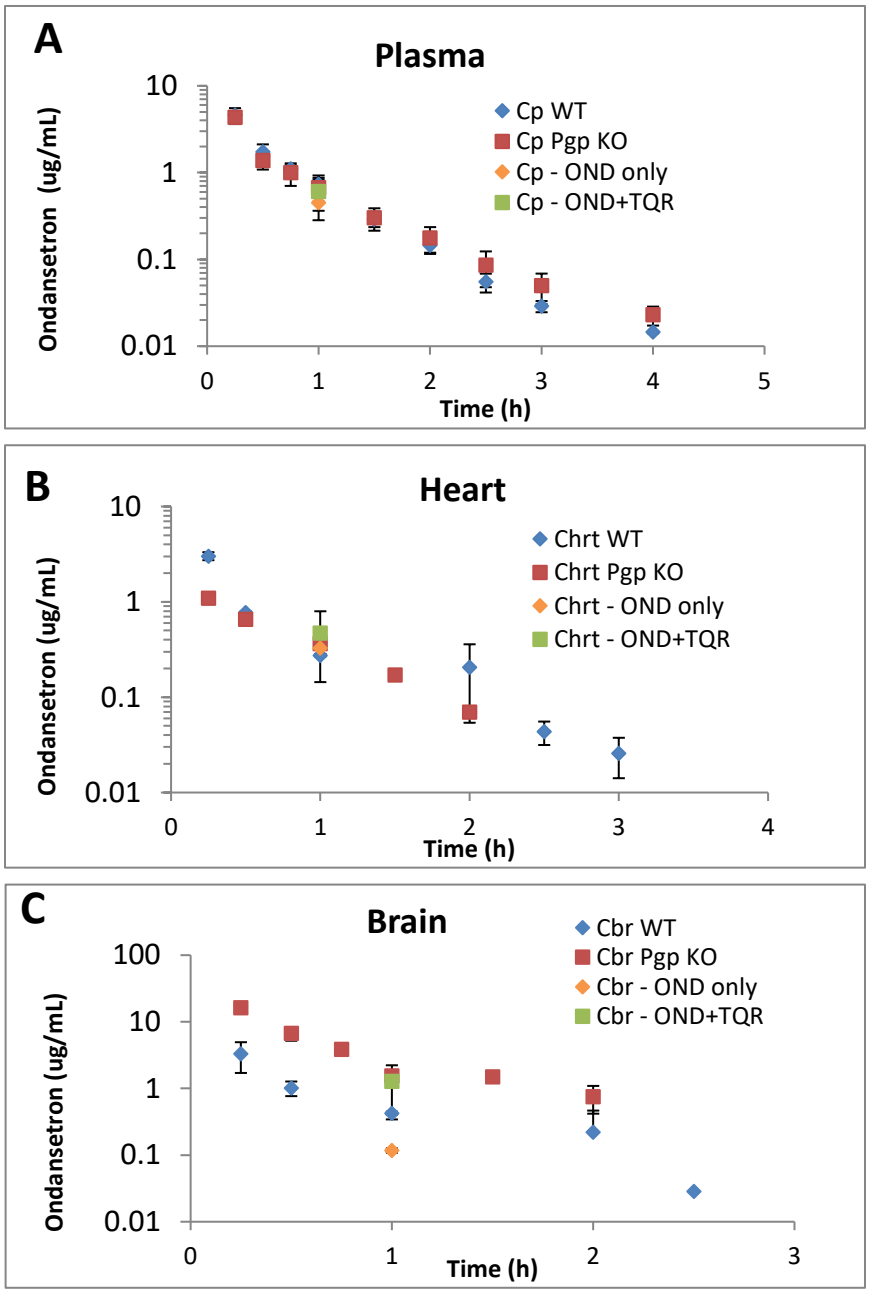


Figure 2.

Tissue concentrations of ondansetron in rats (mcg/mL tissue). Male Sprague-Dawley rats were injected with 10mg/kg IV ondansetron (OND) via a jugular vein cannula and sacrificed at indicated time points. Blue diamonds: concentrations of ondansetron in wild-type (WT) rats. Red squares: concentrations of ondansetron in P-gp knock-out (KO) rats. Orange diamond: single time-point (1h) concentration of ondansetron. Green square: single time-point (1h) concentration of ondansetron; tariquidar (TQR) was administered 30 min prior to ondansetron via a jugular vein cannula.

In addition, a series of experiments were performed to determine the concentration of ondansetron in rat CSF, spinal cord, and brain, performed in wild-type rats, with and without intravenous 7.5mg/kg tariquidar co-administration.

- Plasma to CSF partition coefficient (Cp) of ondansetron, after 10mg/kg dose, in wild-type male rats without tariquidar was 0.27±0.3; plasma to CSF partition coefficient (Cp) of ondansetron, with co-administration of 7.5mg/kg tariquidar was 0.36±0.16

- Plasma to spinal cord partition coefficient (Cp) of ondansetron, after 10mg/kg dose, in wild-type male rats without tariquidar was 1.11±0.4; plasma to spinal cord partition coefficient (Cp) of ondansetron with co-administration of 7.5mg/kg tariquidar was 4.72±2.8

- Plasma to brain partition coefficient (Cp) of ondansetron, after 10mg/kg dose, in wild-type male rats without tariquidar was 1.75±1.2; plasma to brain partition coefficient (Cp) of ondansetron with co-administration of 7.5mg/kg tariquidar was 5.88±5.6

In summary, co-administration of tariquidar resulted in 1.3 fold increase in CSF to blood partitioning coefficient, while in parallel, spinal cord to blood partitioning coefficient increased 4.2-fold, and brain to blood partitioning coefficient increased by 3.4-fold.

Cardiovascular safety data from the first 8 participants in the human pharmacokinetic study per IND 140361 (NCT03809234; 2 participants with 4mg ondansetron, 4 participants with 8mg ondansetron, and 2 participants with 16 mg ondansetron, with and without 4mg/kg tariquidar) demonstrate the following:

- None of the participant had any increase in troponin at any point in the study.
- The QTc interval changes (before to after ondansetron) with and without tariquidar were -2.5±16.1 ms vs 2.9±13.1 ms, respectively (non-significant difference).
- Maximum change in systolic blood pressure was an 8.6±9.7 mmHg increase, two hours after infusion of ondansetron with placebo, and a 5.7± 8.2 mmHg decrease, 1.5 hours after infusion of ondansetron with tariquidar, across doses.
- Maximum change in diastolic blood pressure was an 3.4±7.7 mmHg increase, two hours after infusion of ondansetron with placebo, and an 7.1±9.6 mmHg increase, 105 minutes after infusion of ondansetron with tariquidar, across doses.
- Maximum change in heart rate was an 4.2±13.1 BPM increase, 2 hours after infusion of ondansetron with placebo, and an 12.1±17.0 BPM increase, 105 minutes after infusion of ondansetron with tariquidar, across doses.

The CSF concentrations of ondansetron have been analyzed only in the 8mg dose group.

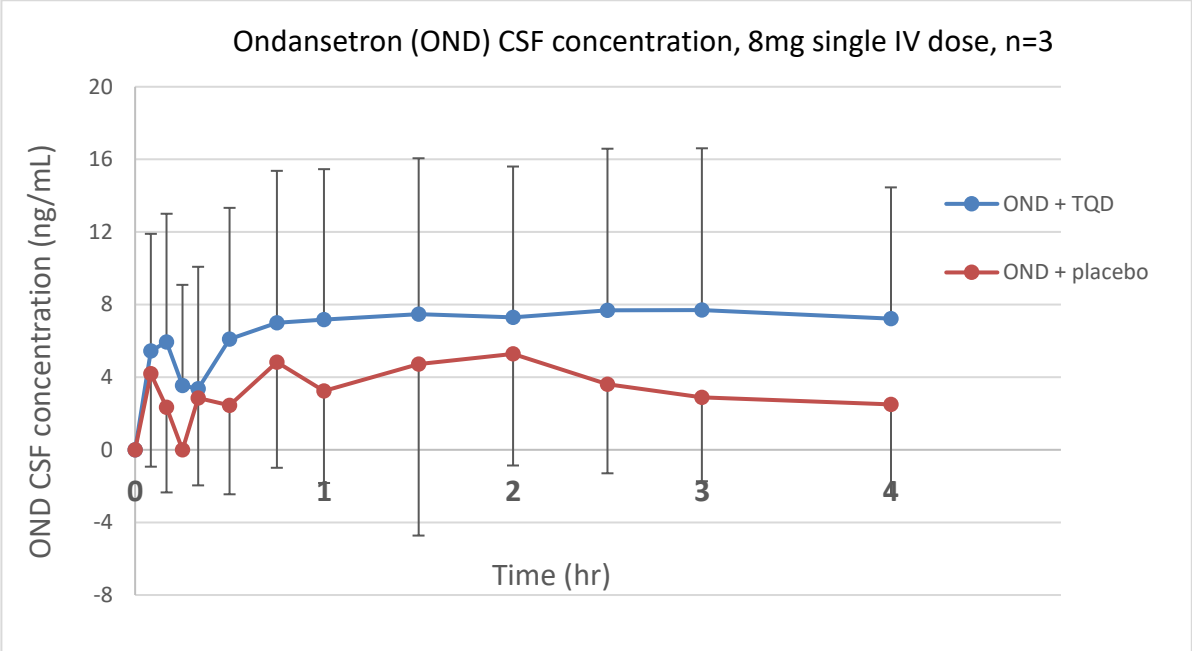


Figure 3. CSF concentrations of ondansetron 8mg iv infusion (over 15 min) with and without concomitant administration of 4mg/kg tariquidar (TQD) in healthy volunteers, (0-4 hours). The area under the concentration time curve (AUC_{0-4}) is 14.1 ng*h/mL for ondansetron administered with placebo (D5W solution), and 28.0 ng*h/mL for ondansetron administered with tariquidar.

Based on rodent data, we expect that the increase in spinal cord and brain exposure with tariquidar co-administration will be higher than the proportionate CSF exposure. However, we don't expect as high as 3-4 fold higher exposure in humans, as Pgp expression in rat blood-brain barrier is substantially higher than in humans.

The tolerability and pharmacokinetic parameters of ondansetron co-administered with tariquidar among patients with neuropathic pain is unknown, and should be evaluated before large-scale efficacy clinical studies.

2.3 Objective

The primary objective of the study is to determine the pharmacokinetics and tolerability of co-administration of 5-HT_3R antagonist ondansetron with a P-glycoprotein inhibitor tariquidar, in patients with neuropathic pain.

Secondary objectives are to assess the change in pain intensity and neuropathic pain descriptors between the two study arms, and to determine psychophysical determinants of analgesic response.

2.3.1 Subject Selection

Patients with neuropathic pain meeting inclusion criteria will be recruited from the Saint Louis community until 28 participants have completed both study sessions.

2.3.2 Inclusion Criteria

1. Age 18-70;
2. Documented diagnosis of neuropathic pain due to damage or disease affecting the peripheral nervous system (e.g. Diabetic neuropathy, Post-herpetic neuralgia, Chemotherapy-induced peripheral neuropathy, Lumbar radiculopathy, or Post-traumatic neuropathy);
3. At least Probable neuropathic pain grading (per Finnerup et al criteria)[1];
4. Pain duration >3 months;

5. Average pain intensity ≥4 on 0-10 numerical rating scale (NRS).

2.3.3 Exclusion Criteria

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

1. Current pregnancy or lactation;
2. Moderate-severe kidney or liver dysfunction;
3. Active cardiac arrhythmias (non-sinus rhythm), Long QT syndrome, or QTc interval >450msec;
4. Congestive heart failure
5. Abnormal troponin values at screening visit
6. Current treatment with MAO inhibitors mirtazapine, SSRI or SNRI antidepressants, except duloxetine up to 60mg/day, escitalopram up to 10mg/day, or citalopram up to 20mg/day;
7. Current treatment with tapentadol, tramadol, or fentanyl
8. Current treatment with P-glycoprotein substrate drugs with narrow therapeutic window, e.g. digoxin.
9. Current treatment with tricyclic antidepressant medications (e.g. amitriptyline, desipramine, imipramine) at a dose >25mg/day
10. Ongoing use of any of the following medications with known effects on Pgp function: carbamazepine, phenytoin, phenobarbital, cyclosporine, clarithromycin, erythromycin, ritonavir, verapamil, rifampicin, St. John's wort;
11. Current treatment with QT-prolonging drugs, and drugs known to have a significant interaction with ondansetron or other P-glycoprotein substrates (see below):
    - Antiretrovirals of Protease inhibitor (e.g. Ritonavir, Saquinavir) or Non-nucleoside reverse transcriptase inhibitors (e.g. Efavirenz, Zidovudine) family.
    - Phenytoin, Carbamazepine, Oxcarbazepine, Rifampin
    - Amiodarone
    - Azole antifungals (e.g. Itraconazole, Fluconazole)
    - Macrolide antibiotics (Erythromycin, Clarithromycin)
    - Cimetidine
    - Lithium
    - Methylene Blue
    - Non-DHP calcium channel blockers Verapamil and Diltiazem
    - First generation antipsychotic medications Thioridazine, Haloperidol, Chlorpromazine, and Pimozide
    - Second generation antipsychotic medications Ziprasidone and Quetiapine
    - Antihistamine Terfenadine,
    - Antidepressants Trazodone and Bupropion
    - Antiarrhythmics Propafenone, Flecainide, and Procainamide
    - Fluoroquinolone antibiotics Norfloxacin, Ofloxacin, and Ciprofloxacin
    - Cisapride
    - Other strong inhibitors or inducers of Cytochromes P450 2D6 or 3A4.
    - Other strong inhibitors or inducers of P-glycoprotein

2.4 Design and Procedures

2.4.1 Study Design

Randomization and Blinding: The study is designed as a prospective, randomized, double-blind, cross-over experiment in patients with neuropathic pain. After signing informed consent and meeting eligibility criteria, participants will receive two 16 mg IV infusions of ondansetron, approximately 3 weeks apart. Participants will be randomized to the order of tariquidar (4mg/kg dose in 500mL D5W) and placebo (D5W solution) co-administration with ondansetron.

2.4.2 Pre-Study Period

Potential participants will be identified from the Pain Management Center of Washington University in St Louis, via ads in the community, Epic-based (and IRB-approved) direct recruitment tools, and through Volunteer for Health (VFH) organization. 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, although this may be done in person at the request of the volunteer.

The following screening information will be collected by phone: age, height and weight, known allergies, complete medical history, neuropathic pain description and intensity. Name and dosing regimen of any over the counter and prescription medications or dietary supplement will be collected as well.

Potential subjects meeting all inclusion and exclusion criteria during the telephone screening will be scheduled for a screening/consent visit at Washington University/Barnes-Jewish Hospital medical center campus. The following will take place at the screening/consent visit, which will occur approximately 1 week before the first treatment study session:

- Verbal discussion of the study procedures, benefits, and potential risks
- Subjects reads, understands, and signs informed consent (subjects will also be provided with a written copy);
- Urine pregnancy test for women of childbearing age;
- Brief history and physical, including vital signs, height and weight measurements, pain history and intensity, and medications;
- Blood draw for troponin, comprehensive metabolic panel (CMP) and complete blood count (CBC); if subject's lab tests results are available within the past 30 days, we will utilize these.
- 12-lead ECG test to rule out QTc interval > 450ms
- Completion of the following questionnaires: Brief Pain Inventory (BPI), Hospital Depression and Anxiety Scale (HADS), and Pain Catastrophizing Scale (PCS), Neuropathic Pain Symptom Inventory (NPSI), Color-Word Matching Stroop Test (CWMST);
- Completion of standardized pain testing: conditioned pain modulation (CPM), wind-up ratio (WUR) to punctate mechanical stimulation, and quantitative sensory testing (QST) for cold, heat, mechanical, and vibration sensitivity.
- Pain diary to track daily pain levels.

Women of childbearing potential with positive urine pregnancy test will be excluded from the study. Women of childbearing potential must agree not to try to become pregnant during the study period and 28 days after study completion. Women of childbearing potential must also use a highly effective birth control method (such as oral contraceptives, condoms, or intrauterine device) throughout and 28 days after the study. Male participants must agree to use a highly effective method of contraception from screening to 90 days after last study drug administration.

Concomitant analgesic use:

Stable doses of analgesics or adjuvant pain medications such as gabapentin, pregabalin, around the clock opioids (with a total daily dose not exceeding 50mg oral morphine equivalent), except those listed in the exclusion criteria, will be allowed, as long as no dose change has occurred 2 weeks prior to the study. PRN analgesic use (NSAIDs, short-acting opioids, acetaminophen) will not be allowed 48 hours prior to study participation. If the participant is unable to abstain from short-term analgesics for the 48 hours prior to the study intervention, the participant can decide whether to reschedule the intervention, or withdraw from the study. The participants can resume their short-acting analgesics 24 hours after the study intervention. The study coordinator will contact the participants by phone once a week during the washout period, and for 3 weeks after the second (crossover) intervention, to address any questions or concerns the participants may have.

SNRI neuropathic pain medications, tramadol, fentanyl and tapentadol will not be allowed, per exclusion criteria.

2.4.3 Study Period

Participants will be studied in the Washington University Pain Center, or in the Clinical Research Unit (CRU) of Washington University School of Medicine, with continued presence of research and nursing personnel. The study will comprise three sessions over an approximately 28-day period (Figure 2A). As previously described, the screening visit will take place within about 7 days prior to the first study visit. The screening visit can be coordinated on the same morning as test day 1.

Intervention:

Each participant will receive two IV infusions of ondansetron, 3 weeks apart, in randomized order. Placebo (D5W) or tariquidar (4mg/kg dose in D5W) will be administered IV over 60 minutes according to randomization schedule. Ondansetron will be diluted in 100mL D5W, and tariquidar will be diluted in 500mL D5W. Starting 30 minutes after the initiation of tariquidar/placebo, a 15-min ondansetron co-infusion will be given, to time its peak brain concentration with potent Pgp inhibition (Figure 3). A 3 weeks interval between the two sessions (> 5 half-lives) will allow the elimination of the first dose of ondansetron and tariquidar. A 12-lead ECG will be repeated prior to the second infusion to confirm that subjects still have a normal QTc and sinus rhythm. Any subject who has a QTc>450ms will not receive the second ondansetron dose and will be withdrawn from the study. A blood sample will be drawn at the 240 min time point for troponin levels. If troponin levels are elevated, the patient will be immediately evaluated by a physician and a cardiology consult will be obtained. Further assessment and treatment of these subjects is described below in section 3, “Management of Intercurrent Events”.

Vital signs (pulse oximetry and 3-lead ECG; non-invasive blood pressure) will be monitored before infusion and approximately 30, 60, 120, 180 and 240 minutes after end of ondansetron infusion. A 12-lead ECG will be performed at baseline and before the participants are discharged.

Adverse effects will be collected by spontaneous participant reporting, but also asked about every 30 minutes.

On each of the intervention dates, women of childbearing potential will undergo urine β-hCG testing, and will be excluded if pregnancy test is positive. Participants will complete a brief pain assessment, as well as WUR and CPM testing at baseline (Figure 2B). Thereafter, two IV catheters will be inserted in participants’ arms. One will be utilized for drug infusion and the catheter in the contralateral arm will be used for serial blood sampling. One 5-ml blood sample will be obtained for P-glycoprotein polymorphism analysis. At study visit 1 and study visit 2, an additional 5-ml baseline blood sample will be obtained for future research with participant consent. Participants will report baseline spontaneous pain (0-10 NRS) and complete a baseline NPSI. Placebo (D5W solution) or tariquidar (4mg/kg dose) will be administered IV over 60 minutes. Starting 30 minutes after the initiation of tariquidar/placebo, a 15-min ondansetron co-infusion will be given, infused via Y-site, to time its peak brain concentration with Pgp inhibition. The intensity of spontaneous pain (on 0-10 NRS) will be assessed every 10 minutes for 60 minutes, and every 15 minutes thereafter for a total of 120 minutes, then every 30 min for a total of 240 minutes. WUR and CPM testing will be repeated 90 minutes after the end of ondansetron infusion (i.e., 105 minutes after the beginning of ondansetron infusion). Participants will record self-reported pain hourly on the study day until bedtime, and daily for 3 weeks thereafter. NPSI pain descriptors will be assessed prior to, and 70 minutes after, the beginning of ondansetron infusion. Additionally, blood samples for ondansetron concentrations will be obtained at baseline (time zero) and approximately at 15, 30, 60, 90, 120, 180, and 240 minutes from the beginning of ondansetron

infusion (See Table 1 for full summary of the measurement schedule.) Plasma will be processed and stored in aliquots at -80°C until analysis. A 3-mL CSF sample (per participant's consent) will be obtained via lumbar puncture between 30 and 180 minutes from the beginning of ondansetron infusion, for the assessment of ondansetron concentration in the CSF using population PK approach as we previously described[31]. Lumbar puncture will not be performed if participant is currently taking anticoagulant treatment or if the participant has a contraindication to the lumbar puncture per investigator discretion.

Participants will return for a follow-up visit approximately 3 weeks after the second infusion, to complete adverse effects assessment, and blood sampling for blood chemistry and blood count.

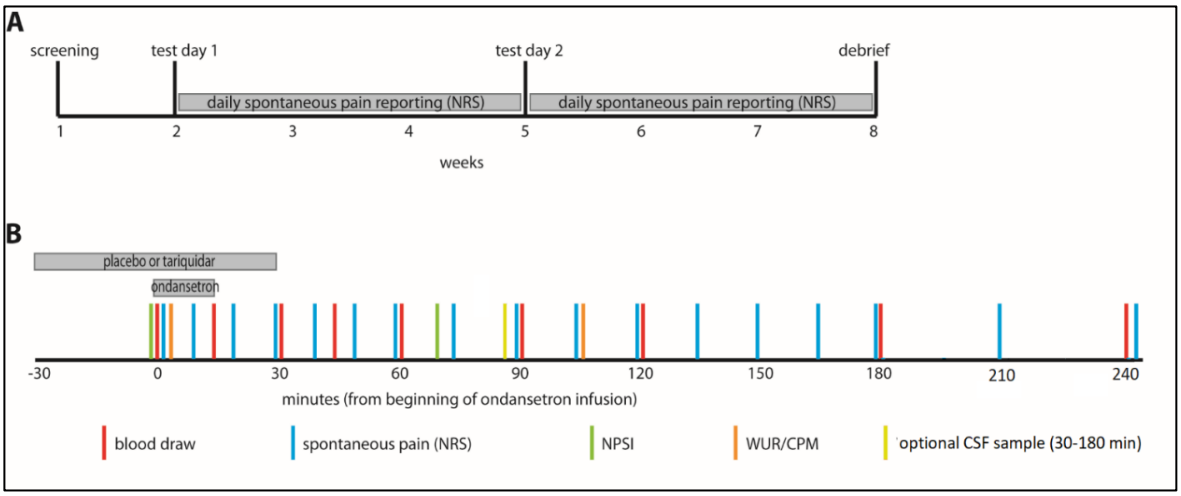


Figure 3. Outline of study design and procedures.
A. Overall crossover design, including screening and testing visits.
B. Testing day procedures. NRS=numerical rating scale; NPSI=neuropathic pain symptom inventory; WUR=wind-up ratio; CPM=conditioned pain modulation

2.4.4 Minimization of Bias

There will be no specific ethnic, racial, or gender background for enrollment. The study is designed as cross-over in order to minimize inter-subject variability. The participants and investigators will be blinded to treatment allocation sequence.

2.4.5 Observations and Measurements

2.4.5.1 Primary Outcome Measures

Primary outcome:
The primary objectives are to evaluate the pharmacokinetics and the tolerability of ondansetron and tariquidar single dose iv co-administration (in comparison to ondansetron administration alone) in patients with neuropathic pain.

Pharmacokinetics:
-Venous blood sampling for pharmacokinetics will be obtained: at 0, 15, 30, 60, 90, 120, 180, and 240 minutes from the beginning of ondansetron infusion.
-A CSF sample will be obtained via lumbar puncture between 30 and 180 minutes from the beginning of ondansetron infusion, for the assessment of ondansetron concentration in the CSF using population PK approach.
-CSF to plasma ratio of ondansetron will be calculated, and compared between the two study arms

Tolerability:
-Spontaneous reporting of adverse effects

-Vital signs (pulse oximetry, blood pressure): before infusion and 30, 60, 120, 180 and 240 minutes after end of ondansetron infusion.
-12-lead ECG: At baseline and prior to discharge after each treatment session.
-Troponin T: at screening and prior to discharge after each treatment session.
-Blood chemistry (CMP) and count (CBC) at baseline and at 3-week time point after each treatment session.
-In patients who are concomitantly treated with SSRI or SNRI medications that are allowed for inclusion (duloxetine ≤60mg/day, citalopram ≤20mg/day, or Escitalopram ≤10mg/day, additional assessments will be performed at the time of vital sign assessments (baseline and 30, 60, 120, 180 and 240 minutes after end of ondansetron infusion) to evaluate confusion, restlessness, dilated pupils, muscle twitching or rigidity, and sweating. This will be performed to identify signs suggestive of serotonin syndrome.

2.4.5.2 Secondary Outcome Measures

Secondary outcome measures include:

1. Change in spontaneous pain intensity (measured on 0-10 NRS) from baseline to 60-120 minutes after ondansetron IV infusion, compared between two sessions with and without tariquidar. The pain severity values obtained at 60, 90, and 120 minutes will be averaged to derive the secondary outcome measure. The values will be compared via paired t-test (or Wilcoxon signed-rank test, if data not normally distributed).
2. Changes in NPSI total score and sub-scores (burning pain, paroxysmal pain, paresthesia/dysesthesia score) will be compared between treatment sessions;
3. The association between baseline Conditioned Pain Modulation (CPM) magnitude (ΔCPM) and the % pain reduction from baseline will be determined by bivariate regression. A significant association between the two measures will be indicative of differential analgesic response as a function of descending pain modulation;

Exploratory analyses will be performed to determine if variables such as sex, weight, baseline wind-up ratio (WUR), peak ondansetron plasma concentration, and P-glycoprotein genotype affect the difference in pain reduction between the two study sessions.
A population PK approach will be used to model CSF concentrations of ondansetron with and without tariquidar co-administration.

Table 1. Study Day Timeline

	Drug		Blood*	PK		NRS	NPSI	CPM	WUR	QST	Questionnaires
Screening	TQD	OND	x	Blood	CSF	x	x	x	x	x	x
Study day (min after ondansetron infusion start)											
Baseline			x	x		x	x	x	x	x	
-30 min	TQD / placebo Infusion										
0 min		OND infusion		x		x					
10 min						x					
15 min				x							
20 min						x					
30 min				x	x**	x					
40 min						x					
50 min						x					
60 min				x		x					
70 min							x				
75 min						x					
90 min				x		x					
105 min						x		x	x		
120 min				x		x					
135 min						x					

150 min						x					
165 min						x					
180 min				x		x					
240 min				x		x					
Hourly after discharge- study day						x					
Post-study day (weeks)											
1						daily					
2						daily					
3			x			daily					

*Blood sample = baseline troponin, comprehensive metabolic panel (CMP) and complete blood count (CBC) at the screening visit, a sample prior to visit #2 (3-weeks after treatment #1), and 3 weeks after treatment #2. An additional blood sample will be obtained for genetic analysis for P-glycoprotein polymorphisms on the first study day; TQD=tariquidar; OND=ondansetron; NRS=numerical rating scale; NPSI=neuropathic pain symptom inventory; CPM=conditioned pain modulation; WUR=wind-up ratio; PK – blood samples for ondansetron pharmacokinetics; QST=quantitative sensory testing.

** CSF sample can be obtained anywhere between 30 and 180 minutes after start of ondansetron infusion.

2.4.6 QST protocol

A QST battery will be applied for more comprehensive sensory characterization of these patients. The QST will follow a standard protocol,[32] and include the assessment of mechanical detection threshold (MDT) with Semmes-Weinstein monofilaments, mechanical pain thresholds (MPT) with standardized weighted metal probes, cold and warm detection thresholds (CDT and WDT), as well as cold and heat pain threshold (CPT and HPT) with TSA-II, and vibration detection threshold (with standard tuning fork). QST tests will be performed in the area of maximum pain and a control, non-painful area.

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.[33,34] The thermode with contact area of 9.0 cm^2 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 Semmes-Weinstein monofilaments (0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256mN). The contact area of the filaments with the skin is of uniform size (<1 mm^2) and texture.

Methods and Background: Standardised Semmes-Weinstein monofilaments 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 [35].

Monofilaments 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 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 "sharp". The corresponding force is used to represent the first MPT value. The stimuli are the applied in the descending order, until the sensation induced by pressure is described as "blunt". The procedure is then repeated a total of 3 times and until a total of 6 values are obtained, from which the mean MPT is determined.

Determination of vibration detection thresholds (VDT)

Equipment: A standard tuning fork.

Methods and Background:

The tuning fork will be applied at the lateral malleolus, and vibration detection threshold (the moment patient stops feeling the vibration of the tuning fork applied to the skin) will be measured on a scale of 1-8. The test will be repeated a total 3 times. VDT is determined as an average of the three measures.

Determination of wind-up ratio (WUR)

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

Methods and Background:

Wind-up ratio (WUR) to punctate mechanical stimulation (i.e. temporal summation) will be measured as another surrogate of descending facilitation. WUR will be assessed by a published protocol.[32,36] In this test a 256mN pinprick probe (Nervetest, MRC systems, Germany) is first applied singularly and then as a series of 10 identical stimuli with 1Hz frequency within an area of 1 cm2. Immediately following the single and 10 stimuli, participants rate their pain on a 0-10 NRS, and a ratio is calculated using these values. This procedure is repeated twice; a geometric 'wind-up' average is calculated from the two ratios.

Determination of conditioned pain modulation (CPM)

Equipment: A thermostat-controlled digital water bath; The Thermal Sensory Analyzer (TSA-II platform - Medoc, Ramat Yishai, Israel)

Methods and Background:

Conditioned pain modulation (CPM) is a surrogate measure of descending inhibition vs. facilitation. CPM efficiency is derived from a patient report of pain intensity following application of a calibrated painful stimulus (called test stimulus) with and without another ongoing unpleasant stimulus (called conditioning stimulus). The intensity of the conditioning and pain stimulus will be determined individually by cold temperature that elicits pain intensity of 30-70 on 0-100 NRS (conditioning), and hot temperature that elicits pain intensity of 60 on 0-100 NRS (test).

The conditioning stimulus will be applied by immersing the non-dominant or non-affected arm in a cold thermostat-controlled water bath. The initial temperature will be set to +8°C. If that temperature elicits pain intensity between 30-70 on 0-100 NRS, the CPM protocol will use +8°C conditioning. Alternatively, water temperature will be adjusted in 2°C increments (lowest will be 2°C) to elicit pain in that range.

The previously determined Pain-60 test stimulus will be applied at the dominant or affected forearm, using a 3x3 cm Peltier thermode connected to TSA-sensory analyzer (Medoc Inc, Ramat Yishai, Israel).

The length of the conditioning is 60 seconds; during the last 30 seconds, the test stimulus is applied. The difference between test stimulus intensity with and without conditioning is defined as CPM magnitude. CPM>0 (i.e. increased pain to test stimulus following conditioning) implies descending pain facilitation.[37,38]

2.4.7 Statistical Methods

All demographic, sensory, analytical and patient-reported data will be captured on case report forms, manually entered to a Research Electronic Data Capture (REDCap) database that will be created for the project, and will be verified by an independent study team member. Plasma and CSF concentrations of ondansetron will be analyzed using a previously validated liquid chromatography method.[39] Non-compartmental PK analysis will be performed to construct the concentration-time curves of ondansetron in human plasma with and without tariquidar.[31] Ondansetron concentration in CSF will be used to evaluate biodistribution of ondansetron to CNS, by the means of CSF to plasma concentration ratio. We do not expect missing outcomes data as all primary and most secondary outcomes will be collected with the participants physically present with the research team. Any missing data will be described, but no data imputation will be attempted. Intergroup comparisons for parametric data will be performed by paired t-test, and for non-parametric data by Wilcoxon signed-rank test for paired samples. McNemar's test will be used to compare paired nominal data, and Fisher's exact test to compare non-paired nominal data.

Sample size:
Change in spontaneous pain reduction (secondary outcome) was used to determine the sample size for the study. The baseline pain intensity (±SD) of patients with peripheral neuropathy we have recruited to various studies over the past two years have been 6.3±2.1. This proof of concept study expects to demonstrate a 30% difference in pain reduction between the two intervention sessions. Given that in a published study of IV ondansetron in NeuP a 20% pain reduction has been observed (although not different from placebo), we assume pain intensity of 5.1+2.1 in ondansetron+placebo group, and expect pain intensity of 4.0+2.1 in ondansetron+tariquidar group at the 60-120 minute timeframe. To determine a significant difference with two-tailed $\alpha<0.05$ and 85% power (effect size 0.52), 28 subjects will be required in this cross-over study. We expect to enroll 28-32 participants to the study to reach 28 evaluable participants.

3.0 Management of Intercurrent Events

3.1 Adverse Experiences
The investigators will closely monitor subjects for evidence of systemic 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. Participants will notify the monitoring physician/RN coordinator of any adverse experience during the study period.
At discharge, subjects will be provided with contact information for team members and instructed to call if any adverse events occur.

3.2 Dose Limiting Toxicities
The dose-limiting toxicities (DLT) for the study are defined as follows:

- New QTc prolongation >60ms greater than baseline, or QTc>450ms
- Troponin T elevation > 0.03 ng/mL
- Syncope
- Sustained increase or decrease in SBP of >30 mmHg systolic or >20 mmHg diastolic
- Sustained vital sign abnormalities
    - HR<40 or >120 beats per minute
    - SpO2 <92%

- ALT/AST ≥3xULN AND total bilirubin ≥2xULN
- AST/ALT ≥8xULN
- Altered mental status (delirium, inability to follow simple commands)
- Persistent visual disturbances

If DLT is observed in >2 participants, ondansetron dose will be reduced to 12 mg for all new participants.
If further DLT is observed in >2 participants receiving 12 mg ondansetron, the dose will be reduced to 8 mg for all new participants.

3.3 Premature withdrawal from a study for a single patient
If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the participant will be withdrawn from the study and the reason(s) for withdrawal documented in the case report forms.

Any participant will be withdrawn from the study in the following scenarios:
- Death
- Anaphylaxis
- Allergic reaction with urticarial, rash or dyspnea,
- Cardiac arrest
- Arial or ventricular fibrillation
- Ventricular Tachycardia
- Syncope
- AV block (2nd degree type II or 3rd degree)
- New QTc prolongation >60ms greater than baseline or >470ms for men or >480ms for women
- Troponin elevation > 0.03 ng/mL
- Sustained increase or decrease in SBP of >30 mmHg systolic or >20 mmHg diastolic
- Sustained vital sign abnormalities
    - HR<40 or >120 beats per minute
    - SpO2 <92%
    - Temperature <36 C or >38°C

- ALT/AST ≥3xULN AND total bilirubin ≥2xULN
- AST/ALT ≥8xULN
- Thrombocytopenia
- Clinically meaningful CBC abnormalities (compared to baseline)
- Altered mental status (delirium, inability to follow simple commands)
- Seizures
- Persistent visual disturbances
- General or specific changes in the patient's condition that render the patient unable to receive further treatment in the judgment of the investigator
- Suspected or confirmed pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- Investigator decides to close the study

If a participant withdraws from the study, the participant 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.

3.4 Adverse experiences and premature termination of the study

If any patient should suffer any serious adverse effect, including but not limited to serious neurologic or cardiovascular dysfunction or a reaction concerning for anaphylactic or anaphylactoid reaction, patient recruitment and randomization will be halted until the etiology of such an adverse reaction can be determined.

If two or more participants suffer any serious adverse effect, the study team will discuss premature termination of the study with Washington University IRB and the FDA.

If more than 7 participants meet the early withdrawal criteria, due to serious or non-serious adverse effects, the study will meet the early stopping criteria.

3.5 Potential Risks

3.5.1 Potential risks for intravenous (IV) catheter placement

Placement of the intravenous catheter can cause a brief, generally mild period of pain lasting for a few seconds at the site of catheter placement. There is the potential that the first attempt at venous cannulation will be unsuccessful and the patient will require multiple attempts to place the IV. Intravenous catheter placement can cause a bruise, though in patients without known bleeding diathesis this is usually mild and self-resolving. Some individuals experience dizziness or lightheadedness. Other complications are rarer. Infection from a cleanly-placed IV is rare, especially given the short anticipated duration of catheter retention. There is the chance of the catheter becoming dislodged from the vein and infiltrating the contents flowing through it into the muscle or subcutaneous fat. As only a minimal amount of fluid will flow through the vein and no caustic or vasoactive substances will be used (except in the case of a rare, potentially serious adverse event), this presents no risk to the volunteer.

3.5.2 Potential risks from lumbar puncture

Lumbar Puncture (LP) is widely used technique of CSF sample aspiration by thin needle from the subarachnoid space of the lumbar dural sac. LP is a major diagnostic tool used in neurological clinics as relatively safe method to obtain CSF sample for further analysis. The important complication of LP is post-lumbar puncture headache (PLPH) developing within a week after LP. In a multisite international trial, 9% of 3868 enrolled patients reported PLPH,[40] with 0.3% patients requiring and epidural blood patch (procedure of introducing autologous blood into epidural space). Other adverse events were non-specific headache (10.2%), back pain (17%), nausea and/or vomiting (2.5%), dizziness 45 (1.3%). Other possible complications may include bleeding as well as temporary or permanent neurological damage, although these are extremely rare. Management of these potential adverse events are described below in section 3.4.

3.5.3 Potential risks from blood collection

The total amount of blood collected during each study visit is approximately 60 mL (about 10% of a standard blood donation), which should not have adverse physiological effects. As described above, the IV catheter placement necessary for blood draws may cause bleeding, bruising, pain, dizziness/lightheadedness, or (very rarely) infection.

3.5.4 Potential risks from ondansetron

Ondansetron is a widely-used drug approved for prevention of chemotherapy-induced, radiotherapy-induced, and postoperative nausea and vomiting. It is commonly used off-label for treatment of nausea and vomiting. The most common reported side effects (~10%) include headache, constipation, and fatigue. Less common (<10%) include drowsiness, diarrhea, fever, anxiety, transient liver enzyme increases, or burning at

injection site. Rare side effects (<1%) include cardiac arrhythmias, ECG changes, dry mouth, anaphylaxis, transient blindness, and hypokalemia.

The FDA has issued a drug safety communication regarding the risk of QTc prolongation and potential progression to torsades de pointes. The risk of cardiac side effects was determined to be dose-dependent with a maximum QTc prolongation by 20msec after 32mg IV administration; consequently, 32mg IV doses are no longer approved. 16mg IV infusion doses (as proposed in this study) are still approved and frequently used for chemotherapy-induced nausea and vomiting. Risk of arrhythmia is increased by comorbidities including: congenital long QT syndrome, congestive heart failure, bradyarrhythmias, or patients taking concomitant medications that prolong the QT interval. All such patients would be excluded by our proposed exclusion criteria. Additionally, in our recent study, none of the 15 subjects who received 16mg intravenous ondansetron (meeting the same inclusion/exclusion criteria as in this study), experienced arrhythmias or any other serious side effects (NCT02901054).

3.5.5 Potential risks from tariquidar

Tariquidar is an experimental drug, a specific 3rd generation inhibitor of P-glycoprotein, currently in development by Avaant Pharmaceuticals Inc. In a Phase I study in healthy volunteers, intravenous tariquidar doses in the range of 0.1-2mg/kg did not cause any adverse effects.[41]

In healthy volunteers, a 2mg/kg intravenous dose resulted in no serious adverse effects. Two of ten subjects receiving this dose developed mild metallic taste during tariquidar infusion. Two subjects who received a 4mg/kg dose experienced light-headedness and conjunctival infection. One subject developed a brief syncopal episode after a PET-scan procedure, resolved without sequelae. A 6mg/kg intravenous dose resulted in mild metallic taste and nausea that resolved after the infusion was stopped.[42]

In healthy volunteers receiving 4, 6 or 8mg/kg intravenous tariquidar doses, no serious adverse events have been observed.[43] Overall, four cases of hematoma and one case of phlebitis due to cannulation, as well as five cases of metallic taste in the mouth occurred. Other adverse drug effects to tariquidar were vertigo (two), bradycardia (one) and headache (one). Heart rate, blood pressure as well as ECG did not significantly change during and after tariquidar administration. No subject had any significant change in blood chemistries (including electrolytes, liver and renal function tests), complete blood count and coagulation over the whole study period. No volunteer discontinued prematurely the study. In one case, for safety reasons the infusion was stopped 20 min before the scheduled end due to moderate orthostatic hypotension and vertigo.[43]

In an additional healthy volunteer study testing intravenous tariquidar doses in a range of 3-8 mg/kg, adverse events possibly/probably related to tariquidar included 1) dysgeusia (changes in the sense of taste) in 6/20 subjects, across 3mg/kg, 4mg/kg, 6mg/kg and 8 mg/kg doses; 2) vertigo in 3/20 subjects receiving 3mg/kg (n=1) and 8mg/kg (n=2) doses; 3) headache (n=1, 6mg/kg dose); and 4) hypotension and bradycardia (n=1, 8mg/kg; infusion stopped after 7.2mg/kg was infused).

Seven clinical studies of the pharmacokinetics and efficacy of tariquidar have been completed in cancer patients. The main use of tariquidar in these clinical trials has been to enhance BBB-penetration and tumor-penetration of Pgp-substrate chemotherapy agents, to improve the efficacy of cancer chemotherapy. In these studies, tariquidar have been shown to increase the severity of known side effects of chemotherapeutic agents, including hematological abnormalities such as anemia and thrombocytopenia. No additional side effects other than those known to be associated with chemotherapy have been identified in patients who have received tariquidar (Tariquidar Investigator's Brochure).

Each ampoule of Tariquidar for injection contains 10 mL of 7.5 mg/mL tariquidar (as the dimesylate salt, in a sterile solution of 20% ethanol/80% propylene glycol with a small amount of hydrochloric acid) for a total of 75 mg tariquidar free base per ampoule. Ampoules will be stored refrigerated (2-8°C) and protected from light. Within 4 hours prior to administration, the tariquidar solution will be diluted aseptically by the Washington University Investigational Drug Service with 5% dextrose in water (D5W) to give a final volume of 250 mL. The diluted solution will be protected from light until infusion is complete.

3.5.6 Potential risks from tariquidar and ondansetron combination

Combined use of tariquidar and ondansetron may change the safety profile of each of drugs. Changes in ondansetron pharmacokinetics such as increased penetration to CNS, is an expected effect of tariquidar. However, there is a potential risk of increased incidence of undesirable effects of ondansetron such as drowsiness, diarrhea, fever, anxiety, transient liver enzyme increases, prolonged QTc, arrhythmias.

Preliminary data from our ondansetron-tariquidar combination study in healthy volunteers suggest no occurrence of arrhythmia or clinically significant QTc prolongation. Most of adverse events were not related with study drugs, but to intrathecal catheterization. QTc changes ater Ondansetron and Ondansetron+ Tariquidar administrations were insignificant: mean QTc change was -2.5 ms (±16.1) after Day 1 and 2.9 ms (±12.9) after Day 2, p=0.67 (see Table 2).

Table 2. QTc values (ms) of subjects on Day 1-3

Subject	Ondansetron dose (mg)	Day 1	Day 2	Day 3
1	4	390	404	417
2	4	434	414	415
3	8	424	430	425
4	8	412	420	405
5	8	399	400	399
6	8	430	397	424
7	16	401	397	406
8	16	395	403	397

3.5.7 Potential risks from thermal testing

Risk of injury related to thermal pain testing is minimal. Thermal testing is widely used and considered 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. The TSA-II thermal devices have FDA 501(k) clearance (K922052). CPM testing is conducted using an ice water bath at 8°C and exposure is limited, thereby posing minimal risk of tissue damage from cold exposure.

3.6 Procedures to Minimize Potential Risks

Drug infusions will be administered in the Washington University Clinical Research Unit under the supervision of the PI and the co-investigators. Subjects will be monitored by study personnel during infusions, and dedicated RN personnel is available 24-hours a day on site.

Inclusion and exclusion criteria, monitoring, and the clinical protocol are designed to ensure that risks are absolutely minimal. Participants 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 at the beginning of the visit that they can alert the investigator any time they experience bothersome side effects. In particular, they will be instructed to immediately report palpitations or dizziness/lightheadedness. Continuous physiologic monitoring (ECG, pulse oximetry, and intermittent non-invasive blood pressure) will be used from the beginning of drug infusion until 2 hours after ondansetron infusion is complete. Medical personnel will be immediately available throughout the visit. The subject can voluntarily withdraw from the study at any time.

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.

3.6.1 Prevention, diagnosis and management of ondansetron-associated arrhythmia

To minimize the risk of ondansetron-associated arrhythmia, subjects will have a 12-lead ECG performed at the screening visit. Subjects with QTc>450msec or any arrhythmia will be excluded. Potential subjects that would be at elevated risk of developing ondansetron-associated arrhythmia (e.g., current use of QT-prolonging medication) are ineligible for this study. The ondansetron dose used (16mg IV infused over 15min) is approved and commonly used with chemotherapy. Subjects will be monitored with telemetry from the beginning of drug infusion until 2 hrs after infusion is complete. If arrhythmias or prolonged QT develop at any time during the study, subjects will be immediately assessed by a physician. If ECG abnormalities are mild (e.g., infrequent premature atrial/ventricular contractions or QTc>450ms but <500ms), subjects will be monitored in the pain management center by nursing and medical personnel for at least 4 hrs. If the signs/symptoms meet the severity criteria described in Section 3.2 ("Premature discontinuation of protocol for a single patient"), the patient will be withdrawn from the study and sent to the Barnes-Jewish Hospital emergency department (ED) for further evaluation, including cardiology consultation or hospital admission, as needed.

3.6.2 Prevention, diagnosis and management of post-lumbar puncture headache (PLPH)

Lumbar puncture and sampling procedure will conducted in a way to minimize the effect of risk factors contributing PLPH development. Particularly, an atraumatic needle of 25 G diameter will be used for LP procedure. Passive withdrawal of CSF is known as associated with a lower risk for headache, therefore active withdraw with syringe will be avoided left only for rare cases.

Subjects will be asked to notify clinical personnel immediately if they develop a headache while in the clinical research unit. Additionally, the periodic nursing assessment (see supplementary document) will include questions about headache. If a participant endorses new-onset headache, the anesthesiologist will be notified. The anesthesiologist will perform a brief history and physical exam to determine whether or not the symptoms suggest PLPH. If PLPH is likely and symptoms are mild, conservative treatment will be initiated. The participant will be encouraged to lie in a comfortable position and drink fluids. The subject will also be offered analgesics including acetaminophen/butalbital/caffeine (e.g. Fioricet®), ibuprofen, or acetaminophen alone. IV fluids or medication will be reserved for subjects who cannot tolerate oral administration due to nausea/vomiting. If symptoms are severe (e.g. uncontrollable nausea/vomiting, severe subject distress), the subject will be removed from the study and epidural blood patch will be offered (procedure described below).

Discharge paperwork will include clear descriptions of PLPH and instructions about whom to contact in the case of headache. If a subject reports likely PLPH by contacting study personnel or during a follow-up phone call, symptom severity will be assessed. Conservative treatment (as described above) will be offered for the first 24-48 hrs. If unresolved, the participants will be brought to the Clinical Research Unit for further evaluation or treatment per discretion of the investigators which could include a potential epidural blood patch procedures, performed by the anesthesiologists on the study team.

Epidural blood patches are commonly performed by our Acute Pain Service. Sterile technique (including chlorhexidine swabs, caps, masks, and sterile gloves) is used at all steps by all personnel involved. First, the epidural space is located using a standard Tuohy needle and loss-of-resistance technique. Next, 20ml of the subject's blood is drawn and then injected through the Tuohy needle. Injection is stopped if the subject complains of pressure or paresthesia that could indicate compression of the cauda equina.

3.6.3 Prevention of drug-drug interactions between study drugs and concomitantly used antidepressants

Citalopram, Escitalopram and Duloxetine are not known to produce clinically meaningful interactions when combined with potent P-gp or CYP3A4 inhibitors. When tested specifically in combination with P-gp inhibitors such

as simeprevir, cyclosporine, and verapamil, meaningful interactions were NOT identified for escitalopram and duloxetine[44-46]. Given that tariquidar is a selective P-gp inhibitor (and possibly weak CYP3A4 inhibitor), we do not expect any meaningful interactions with citalopram, escitalopram, or duloxetine.

Table 3 outlines the potential of drug interactions between SSRI/SNRI antidepressants in the context of use with P-gp inhibitors, and served as a basis for inclusion/exclusion of particular SSRI/SNRI antidepressants.

Table 3. Potential of drug interactions between SSRI/SNRI antidepressants and P-gp inhibitors.

SSRI	**FDA-approved maximum dose (mg/day)**	**Known P-gp substrate**	**Known CYP3A4 substrate**	**Known / Clinically relevant interactions with other P-gp inhibitors**	**Clinically-relevant interactions with CYP3A4 inhibitors**	**Comments on QT interval prolongation**
Citalopram	40	**No**[47,48]	Yes, CYP3A4 and CYP2C19[49]	None found (extensive search in PubMed, Lexi-Comp, and IBM Micromedex)	No. ketoconazole co-administration did not significantly affect the pharmacokinetics of citalopram [1]	Dose-dependent QT prolongation [47] [50]
Escitalopram	20	**No**[48] [51]	Yes, CYP3A4, CYP2D6, and CYP2C19	None found (extensive search in PubMed, Lexi-Comp, and IBM Micromedex)[49]	No. Administration of ritonavir (potent CYP3A4 inhibitor) did not affect the pharmacokinetics of Escitalopram [52]	No clinically relevant QT prolongation vs placebo [52]
Paroxetine	60	**Possibly**[53] [54]. However, may be a weak inhibitor of P-gp [55]	No. CYP2D6 and unchanged excretion to urine	No	No. Also, paroxetine does not meaningfully inhibit CYP3A4[56]	Likely rare, only a few cases reported [57]
Fluoxetine	80	**Conflicting data** [53,54] [58]	Yes. Also possible inhibitor of CYP 3A4. Although overall CYP3A4 inhibition with fluoxetine is likely not clinically meaningful [44], some potential interactions have been reported with CYP3A4 substrates.[59]	No	Verapamil (likely due to Fluoxetine's inhibition of verapamil metabolism [44])	Fluoxetine has been associated with potential increase in risk of QT interval prolongation [50]

Sertraline	200	**No**[60,61]. However, may be a weak inhibitor of P-gp[55]	Unclear. Multiple sources of data suggest 1st pass metabolism and N-demethylation, but specific enzymes are not well understood[60,61], possibly suggesting CYP2C19 and CYP2B6	None found (extensive search in PubMed, Lexi-Comp, and IBM Micromedex)	No	Likely not significant[62]
SNRI	**FDA-approved maximum dose (mg/day)**	**Known P-gp substrate**	**Known CYP3A4 substrate**	**Known / Clinically relevant interactions with other P-gp inhibitors**	**Clinically-relevant interactions with CYP3A4 inhibitors**	**Comments on QT interval prolongation**
Duloxetine	120	**No**[63],[58]	No. Metabolized primarily by CYP1A2 and CYP2D6	None found (extensive search in PubMed, Lexi-Comp, and IBM Micromedex)[49]	None found	No clinically relevant QT prolongation vs placebo [63]
Venlafaxine	225	**Possibly** [53,54]	Yes. Also metabolized by CYP2D6 (major). Venlafaxine does not meaningfully inhibit CYP3A4 [64]	Interaction with ketoconazole, likely due to CYP3A4 rather than P-gp inhibition. Minimum interference with P-gp activity in vitro [64]	Clinically meaningful interaction with ketoconazole, likely because 3A4 inhibition [46]	QT prolongation rare, likely happens only with very high doses / overdose[65,66]
Desvenlafaxine	400mg, but doses >50mg not recommended	**No**[67]	Yes (minor). Also excreted renally unchanged (45%). Desvenlafaxine does not meaningfully inhibit CYP3A4 [64]	Unlikely to be affected[67]	Ketoconazole can increase plasma concentrations of desvenlafaxine. Potential interaction with potent CYP3A4 inhibitors.	No clinically relevant QT prolongation vs placebo[67]

For patient safety, we will limit maximally allowable doses of **citalopram**, **escitalopram** and **duloxetine** in enrolled participants to 50% of FDA-approved maximum dose, eg, 20mg for citalopram, 10mg for escitalopram and 60mg for duloxetine.

Due to possible interactions with other SSRI/SNRI medication, we will exclude patients taking other SSRI/SNRI medications such as paroxetine, fluoxetine, sertraline, venlafaxine, and desvenlafaxine.

3.6.4 Prevention, diagnosis and management of other lumbar puncture-related complications

The potential complication of epidural hematoma is exceedingly rare in patients with normal coagulation[40]. To minimize the risk of epidural hematoma, subjects will be asked over the phone and at the in-person screening visit about a history of clotting disorders, easy bleeding/bruising, liver disease, or use of anticoagulants. A complete blood count will be drawn during the screening visit to rule out thrombocytopenia. Any potential subject with abnormalities on history or lab work will be excluded from the study.

To minimize the risk of infection or nerve damage from lumbar puncture, the procedure will be done by experienced anesthesia personnel (board-certified or board-eligible anesthesiologists) under sterile conditions. Specifically, the subject's lumbar region will be sterilized with a chlorhexidine swab, a sterile drape will be placed, the anesthesiologist will wear sterile gloves, all personnel in the room will wear a cap and mask, and a dressing will be applied after LP. Subjects will be instructed to notify the anesthesiologist of the occurrence and resolution of any paresthesias during LP procedure. If symptoms are severe (e.g., severe pain, motor deficits) the subject will be sent to the BJH ED for further evaluation including neurology consultation, imaging, or hospital admission, as needed. Subjects with temporary, minor paresthesias during placement may continue with the study.

3.7 Data and Safety Monitoring Plan

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, and commensurate with the risks of the proposed study, we will use an Independent Data Safety Monitoring Board (DSMB) for data safety and monitoring for this study. The potential risks are attributable to the 5-HT_3R antagonist ondansetron, and the Pgp efflux transporter inhibitor tariquidar. The Independent DSMB will consist of an anesthesiologist, a Neurologist, and an independent Biostatistician. This DSMB will review the annual summary of adverse events, prior to data and safety monitoring report submission to the Washington University IRB. In addition, the board will review all reports of a Serious Adverse Event, or an Unexpected Adverse Event.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO according to institutional guidelines.

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, who is a GCP-certified pharmacist with several years of experience in the conduct of human studies; three board-certified anesthesiologists pain management specialists as co-investigators, all of them with extensive clinical experience and research experience.

4.2 Sources of Materials

Subjects will be recruited from the greater Saint Louis area via their Washington University Pain Management Center physicians, flyers, IRB-approved Epic direct recruitment tools, and the Volunteer for Health organization. Data on comorbidities and concomitant medication use are provided by subjects. Specimens include blood obtained exclusively for determining ondansetron concentration, and an additional blood sample is obtained for determining

single nucleotide polymorphisms in P-gp and OCT1 transporters. Other data including quantitative sensory testing are obtained exclusively for research purposes.

4.3 Recruitment and Informed Consent

Participants will be recruited through their Washington University Pain Management Center physicians, flyers, and through the Volunteers for Health organization. Interested subjects will contact the investigators. We may also screen the clinic schedule of the Washington University Pain Management Center, to identify potentially eligible subjects. We will then approach or contact them to ask for their interest in the study. 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 study team provides all study descriptions, informed consent, and answers all questions. 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

Study subjects could potentially experience improvement in neuropathic pain. Society could benefit from development of a novel treatment for neuropathic pain.

4.5 Inclusion of Women

Both women and men suffer from neuropathic pain and thus will be included in this study.

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 years of age will not be studied in this investigation. Neuropathic pain is uncommon in this age group, and including children may expose them to an unnecessary risk without the benefit of generalizability of the results.

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