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Study Title: Neostigmine and Glycopyrrolate by Iontophoresis to Induce Bowel Evacuation

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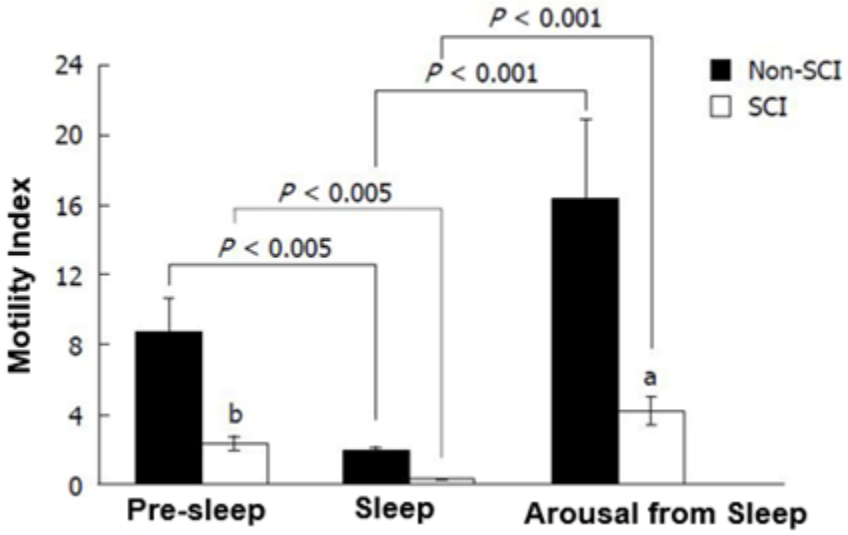
ABSTRACT:
Background: Persons with spinal cord injury (SCI) have neurogenic bowel disorders which is associated with significant morbidity. The negative impact of bowel complications is often at the top of the list of problems reported by persons with SCI. Despite the magnitude of the problem of bowel dysfunction in persons with SCI, and the associated reduction in quality of life, this condition has yet to be effectively treated. The investigators have developed a novel dual drug combination to elicit a safe and predictable bowel evacuation (BE).
Objectives:
Specific Aim: To determine a lower effective dose to induce BE by transcutaneous administration of NEO by ION (primary objective).
Hypothesis: An effective dose of neostigmine (NEO) to achieve BE will be lower than the standard dose previously used for intravenous and transcutaneous administration of these agents by iontophoresis (ION).
Setting: Patient enrollment and study procedures will be performed at the James J. Peters Veterans Affairs Medical Center (JJPVAMC).
Design: On Day 1, NEO (0.02 mg/kg) and GLY (0.004 mg/kg) will be administered IV. During the second visit, at least 24 hours later, subjects will receive NEO (0.07 mg/kg) + GLY (0.014 mg/kg) by transdermal administration by use of a wired iontophoresis system, which will be repeated to confirm reproducibility. Pharmacokinetics will be obtained over 2 hours and drug side-effects, recorded. Because almost all participants in prior studies have had BE with the dose of NEO currently employed, decreasing doses of NEO will be administered until two dose reduction titrations are performed (to 50% of the standard dose).
Participants: Thirty (30) participants will be enrolled.
Outcome measures: Presence or absence of bowel evacuation, time to bowel evacuation, consistency (Bristol stool scale), and quantity (by weight). The presence/number of cholinergic and anti-cholinergic side-effects on a standardized point scale will be determined, as well as their severity (e.g., mild, moderate, or severe). Heart rate, blood pressure, and pulmonary mechanics (airway patency by impulse oscillation system) will be recorded at baseline and sequentially at intervals the initiation of ION (at 5, 10, 15 20, 30, 45, 60, 90, and 120 minutes). Blood will also be drawn at the aforementioned time intervals for pharmacokinetic analysis.
Funding Source: Congressionally Directed Medical Research Programs (Grant # SC180166)

Background:

Neurogenic Bowel Dysfunction after Spinal Cord Injury

Persons with spinal cord injury (SCI) have neurogenic bowel disorders associated with significant morbidity.[1] In a high proportion of individuals with SCI, it often takes several hours 3 to 4 times a week to induce a partial bowel evacuation, despite adherence to regimens that include meticulous dietary habits with regard to composition, quantity, and timing of food consumption, stool softeners, fiber content of meals, fluid consumption, suppositories, laxatives, enemas, and digital rectal stimulation. In self-reported surveys, the negative impact of bowel and bladder issues is often at the top of the list of problems that confront persons with SCI. Thus, the bowel care regimens that are available to persons with SCI are often inadequate and result in significant morbidity. Despite the magnitude of the problem of bowel dysfunction, and the associated reduction in quality of life, this condition has yet to be effectively treated.

Figure 1



Although the enteric nervous system remains intact after SCI, our group demonstrated markedly reduced motility of the colon in persons with SCI.[2-4] The motility index (MI; product of the mean amplitude and percent activity) at rest (basal) and in response to a standard test meal (post meal) was significantly ($p<0.01$) depressed in those with SCI compared to controls; MI in the SCI group: 2.6±0.6 (basal) vs. 4.6±0.7 (post meal) mm Hg; MI in the able-bodied control group: 8.3±3.2 (basal) vs. 13.3±5.4 (post meal) mm Hg.[2] The mean amplitude of the peristaltic waves was significantly lower in the group with SCI than in the control group (31.2±1.9 vs. 44.8±7.3 mm Hg). The number of waves per hour was also significantly lower in the SCI group than the control group (70.0±9.1 vs. 119.1±22.2). [2] Upon arousal from sleep, the MI was also markedly depressed (Figure 1) in SCI. Of note, the high amplitude propagating contractions (e.g., the forceful contractions that drive colonic contents toward the rectum) that were present in able-bodied control subjects (solid bars) were not observed in subjects with SCI (absence of open bars pre-sleep, during sleep, or upon arousal) (Figure 2).[3]

Colonic transit time was assessed by radiopaque markers and was found to be greatly lengthened in those with SCI (Figure 3).[3] Confirming the finding with radiopaque markers, total colonic transit time (mouth to anus) with smart pill technology was significantly decreased (52.3±42.9 vs. 14.2±7.6 hours).[4]

To develop a successful bowel care routine in an individual with SCI is approached empirically. Medications that are employed in the management of bowel dysfunction may be divided into those administered by mouth or per rectum or by their pharmacological mechanism of action. The categories of agents include bulk-forming agents, stool softeners, and laxatives. Various direct bowel wall stimulants have been used, and include senna preparations by mouth or per rectum, castor oil, magnesium preparations by mouth, sodium phosphate/biphosphate by mouth. Even after applying these measures to induce a regular bowel evacuation, persons with SCI frequently have incomplete and unpredictable bowel evacuation, which

Figure 3

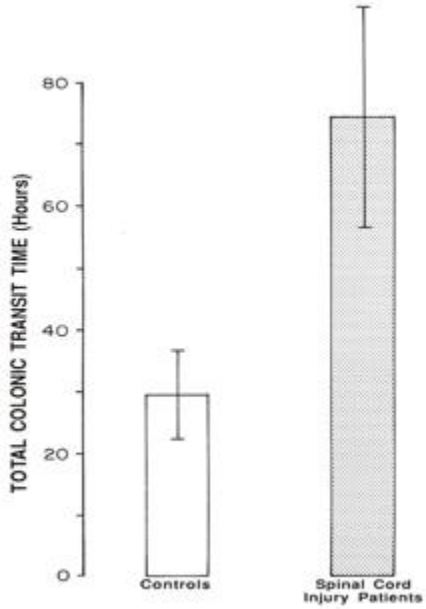
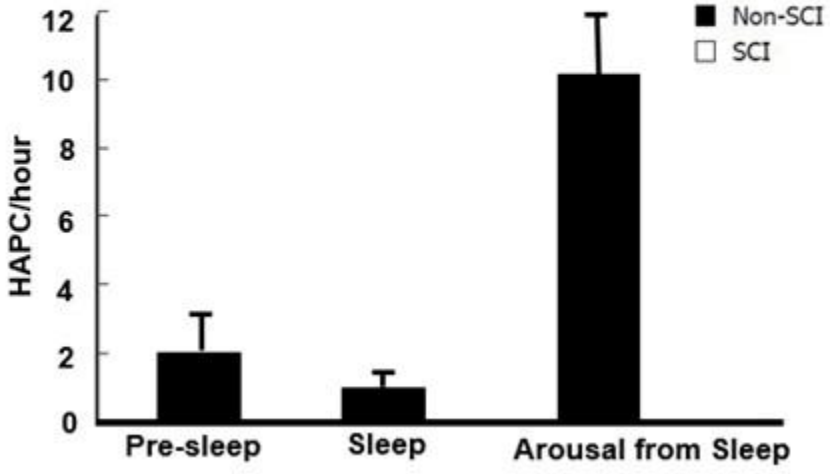


Figure 2

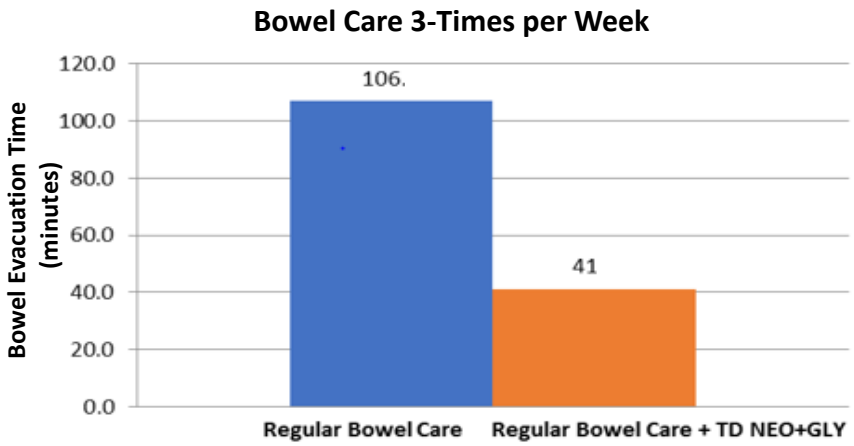


may result in discomfort, autonomic dysreflexia, and/or stool incontinence. The inability to empty the colon predictably and completely results in a high risk of incontinence. Fecal incontinence is a source of humiliation, lost time, added effort, additional expense, and often increased attendant support. The possibility of bowel accidents is often provided as a reason that those with SCI remain homebound and shy away from activities in the community.

Efficacy and Safety of NEO and GLY to Elicit Bowel Evacuation in Veterans with SCI

Our work for the past decade with a prokinetic agent (e.g., neostigmine) was the first to demonstrate a stimulated, predictable and complete colonic bowel evacuation. The drug combination of neostigmine (NEO; a cholinergic agent) and glycopyrrolate (GLY; a selective cardiopulmonary anticholinergic agent) was demonstrated by intravenous (IV) administration to predictably stimulate bowel evacuation without serious adverse cholinergic effects on the heart and lung.[5,6] This dual drug combination was also efficacious if administered intramuscularly (IM).[7] The effectiveness and reliability of this dual pharmacological approach to induce bowel evacuation is far greater than that of oral or rectal cathartics. However, the parenteral administration of drugs often meets resistance from patients, IV administration is difficult to perform outside of a medical setting, and the IM administration is associated with pain, hematomas (may impair mobility and transfers if administered in the upper extremities), and possibly autonomic dysreflexia (due to an IM injection being a noxious stimulus if administered below the level of SCI). An alternative mode of administration of NEO and GLY was sought and identified; our group has shown that NEO + GLY may be effectively delivered by transdermal administration by iontophoresis (ION)[8]. Subsequently, using a modified protocol, our rate of success to induce a bowel evacuation is approximately 90% compared to a 40% success rate reported in our March 2018 article[8]. In recent work to determine the efficacy and tolerability of this approach, subjects were administered transcutaneously by ION NEO + GLY 3-times a week for two weeks; the length of bowel care sessions was reduced dramatically with transcutaneous administration compared to the regular bowel care routine (Figure 4; unpublished observation). However, administration of the dual drug combination transcutaneously in 10 subjects resulted in the following untoward side-effects: dry mouth was present in 80%, muscle fasciculations in 30%, and dizziness in 20% of subjects tested; the average duration of these side effects ranged from 26 to 2 minutes (unpublished observation). Because the bioavailability of the two agents may differ significantly when delivered transcutaneously by ION versus by IV, the appearance of side-effects would be anticipated to depend upon the mode of administration, the absolute dose of the drugs, and the ratio of NEO to GLY.

Figure 4



The pharmacokinetics of the transdermal drug delivery of these agents has not been addressed. The question arises as to the optimum concentrations of the component medications to achieve the endpoint of predictable bowel evacuation without eliciting cholinergic side-effects from NEO administration on the heart (e.g., bradycardia) and lung (e.g., bronchoconstriction) but without producing anti-cholinergic side-effects from GLY administration.

<u>Specific Aims</u>:
In persons with SCI:

Specific Aim (1): To determine a lower effective dose of NEO to induce bowel evacuation by transcutaneous administration by ION.
Hypothesis (1): Almost uniform bowel evacuation was observed at the current dose of NEO (0.07 mg/kg) delivered by transcutaneous administration by ION ("ceiling effect"); as such, the minimum effective dose of NEO to achieve this endpoint should be lower. Because of differences in absorption of drug, space of distribution, and biological effect, the effective dose of drug will vary to a certain extent from person to person.

Study Design:

Subject Availability, Demographics, and Enrollment:

There are approximately 370 patients with chronic SCI who are followed at the James J. Peters Veterans Affairs Medical Center (JJP VAMC). The SCI Service at the JJP VAMC is a 40-bed inpatient unit. Each year, ~140 Veterans with SCI are admitted as inpatients to our VA facility. Annually,~350 Veterans are treated in our facility's outpatient SCI clinic. Approximately 50% of SCI patients have a motor-complete injury [American Spinal Injury Association Impairment Scale (AIS) A & B obtained by examinations using the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)], with the majority of the remaining patients having AIS C classification and being non-ambulatory. The patients with SCI have the following characteristics: approximately one-third has paraplegia and two-thirds have tetraplegia; 80% are male; 60% are white and 40% are African-American. Patient recruitment will occur from prior participants in our Centers' research studies and the outpatient clinics. Subjects will be informed verbally and in writing of the purpose of this study. Informed consent will be obtained from all subjects who agree to participate or, if not able to provide informed consent, will be obtained from their legal surrogate. Subjects will be free to withdraw their consent at any time. Subject travel costs and a stipend for participation will be provided by the grant.

Records will be kept confidential by removing the name of the subject from all data and assigning each subject a random identification number which will be recorded on all subject data sheets and linked by identifies to master key that is appropriated secured. An investigator or research coordinator will enroll subjects, schedule travel, coordinate blood/urine laboratory studies, collect data, and coordinate the responsibilities of all study subjects.

Inclusion Criteria:

(1) Male or female; (2) Age ≥18 years; (3) Chronic SCI (>1 year post injury); and (4) You have documented constipation/difficulty with bowel evacuation and/or experience pain, straining, or fecal incontinence

Exclusion Criteria:

(1) Previous adverse reaction or hypersensitivity to electrical stimulation; (2) Known sensitivity (prior reaction or allergy) to neostigmine or glycopyrrolate; (3) History of mechanical obstruction (physical blockage) of the GI or urinary tract (e.g., due to scar tissues forming after surgery, gallstones); (4) Myocardial infarction (heart attack) within 6 months of trial; (5) Malignant and/or uncontrollable hypertension (high blood pressure) defined by a blood pressure reading of 160/100 mmHg or higher with or without taking 3 or more different classes of anti-hypertensive medications (drugs used to treat high blood pressure); (6) Organ damage (heart & kidney) and/or transient ischemic attack/cerebrovascular accident (TIA-CVA, or stroke) as a result of hypertension; (7) Known past history of coronary artery disease or bradyarrhythmia (slow heart rate); (8) Symptomatic orthostatic hypotension (low blood pressure with possible dizziness/fainting); (9) Deep brain stimulation; (10) Pregnancy (women who are sexually active and of childbearing potential must utilize a method of contraception and agree to maintain a contraceptive method until completion of the study); (11) Lactating, nursing females; (12) Inability to provide informed consent determined by Montreal Cognitive Assessment Test (MoCA) score of 20 or less. This test is used to detect mild cognitive impairment; (13) History of ingrown hair folliculitis (inflammation of hair follicles); (14) Concurrent illness (with or without concurrent fever), such as lower respiratory illnesses, increased mucous/secretin production, congestive heart failure (CHF), or pneumonia; (15) Currently taking the following

medications: Bethanechol, Chloroquine, Colistin, Penicillamine, Lithium, Methylcellulose, Trimeprazine, Verapamil, Phenothiazines, Sparfloxacin, Amitriptyline, Doxepin, Imipramine, Potassium chloride, Saquinavir, Dronedarone, Cisapride, Bepridil, Terfenadine, Amiodarone, Ziprasidone, or any medication(s) that could result in adverse reactions with neostigmine and/or glycopyrrolate, as determined by a study physician; (16) Currently taking cholinesterase inhibitors, such as those for Parkinson's Disease (PD) or dementia (rivastigmine, donepezil etc.), or medication with anticholinergic activity, such as anti-depressants; (17) Myasthenia gravis; (18) EKG abnormalities such as bradycardia, prolonged QTc interval, axis shift, bundle branch block, Wolff-Parkinson-White Syndrome (WPW syndrome), 2nd and 3rd degree heart blocks etc. (determined at screening 12-lead EKG); (19) Chronic gastrointestinal (GI) disease such as inflammatory bowel disease (IBD), irritable bowel syndrome with constipation (IBS-C), or other causes of difficulty with stool evacuation such as hypothyroidism (underactive thyroid); (20) Fever (as an isolated symptom without "illness"), or who may be exposed to high environmental temperatures; and (21) Concurrent participation in a research study.

HRPO Reporting:

(1) The following study events will be promptly reported to the U.S. Army Medical Research and Development Command (USAMRDC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), by facsimile (301-619-7803), or mail to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000. All unanticipated problems involving risk to subjects or others.
(2) Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies.
(3) Any instances of serious or continuing noncompliance with the federal regulations or IRB requirements.
(4) The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research.
(5) The issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any government regulatory agencies.

Independent Research Monitor

The Research Monitor, Noam Y. Harel, MD, PhD, is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

A baseline EKG will be obtained to exclude participants with any EKG abnormalities. An IV line will be inserted; study drugs will be administered through the IV catheter. IV NEO (0.02 mg/kg) and GLY (0.004 mg/kg) will be administered to potential participants. At the start, a 30 second IV push of NEO will be followed by a NS flush (12mL), and two minutes after the start of the first IV push, there will be a 30 second IV push of GLY, followed by a NS flush (12 ml). The pharmacokinetics of IV NEO has been described (Figure 5). Note that after the initial phase of equilibration (first exponential decline), the disappearance of NEO is linear over the next 60 minutes, and would be expected to continue to fall over the ensuing 60 minutes, approaching baseline.[9] Thus, it would be anticipated that, regardless of the mode of administration, the$T_{1/2}$ of the second phase of disappearance of NEO from the circulation would be quite similar; only the time to peak will vary with the mode of administration, and time to peak would be expected to be delayed by transcutaneous administration compared to that of IV administration. If any subjects experience hyperactive bowel sounds without a bowel evacuation following the administration of NEO and GLY, digital rectal stimulation will be performed to relax the anal sphincter and induce a bowel evacuation at the discretion of the PI, Dr. Cardozo. The investigators have previously reported that that approximately 75% of individuals who receive IV administration of these agents will respond with bowel evacuation.[8]

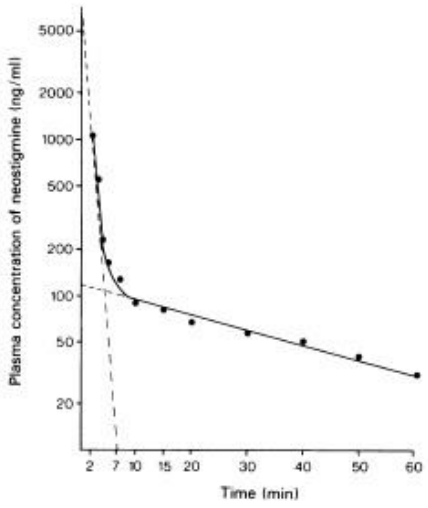


Figure 5

TABLE OF STUDY EVENTS (Screening)*											
Time Points (in minutes)	BL	0	5	10	15	20	30	45	60	90	120
EKG	x										
NEO+GLY administration (IV)		x									
Blood collection (2ml per vial)	x	x	x	x	x	x	x	x	x	x	x
Heart rate (HR)	x	x	x	x	x	x	x	x	x	x	x
Blood pressure (BP)	x	x	x	x	x	x	x	x	x	x	x
Pulse Oximetry (SPO2)	x	x	x	x	x	x	x	x	x	x	x
Impulse oscillation (IOS)	x	x	x	x	x	x	x	x	x	x	x
Assessment of bowel sounds	x	x	x	x	x	x	x	x	x	x	x
Symptoms Survey	x	x	x	x	x	x	x	x	x	x	x

*Digital rectal stimulation will be performed at the discretion of the PI, Dr. Cardozo.

<u>Intravenous Neostigmine and Glycopyrrolate (Study visit 1)</u>
Drug Concentration
Neostigmine Methylsulfate Inj., USP 1mg/mL, 0.02 mg/kg (Avadel Pharmaceuticals, Chesterfield, MO)
Glycopyrrolate Inj., USP 0.2 mg/mL, 0.004 mg/kg (American Regent, Inc., Shirley, NY)

The maximum dose of NEO is limited to 10.0 mg and the dose of GLY to 2.0 mg per subject per administration.)

Study 1: **Transdermal Administration of NEO and GLY at the Standard Concentrations and Ratio**

Trial 1: Subjects will progress to receive the standard dose previously reported and employed for these agents [NEO (0.07 mg/kg) + GLY (0.014 mg/kg)] by transdermal administration by use of a wired ION system (Figure 6).[8] We have consistently demonstrated that NEO and GLY can be delivered into the systemic circulation by transcutaneous route by ION to induce a safe and predictable bowel evacuation in persons with SCI. If digital rectal stimulation was utilized in the screening session, this method will be utilized for the remaining sessions as needed at the discretion of the PI, Dr. Cardozo. In the anesthesiology literature, a ratio of NEO to GLY of about 5 to 1 has been employed in clinical situations.[10,11] Participants will be requested not to have bowel care for at least 1 day prior to the study. The subject will assume his/her normal bowel evacuation position until a bowel movement occurs. Privacy draping and privacy will be provided at the time of bowel evacuation. The subjects will be monitored for 120 minutes. A minimum of two research personnel will be present during the study visit to record all of data and perform the tasks required.

Figure 6

From our recent work, it is extrapolated that 85 to 90% of participants will have a bowel evacuation to transdermal administration of NEO by ION. The pharmacokinetics of only those who have a bowel evacuation to NEO will be used in the calculation to determine the peak plasma concentration (PPC) and area under the curve (AUC) of NEO and GLY. The PPC and AUC for the non-responders to NEO will be analyzed separately; it is speculated that the PPC of NEO for the non-responders will be blunted—that is, at least below the mean value of the PPC for NEO administration.

The absorption of study agents is, to a certain extent, subject-specific because of differential absorption through the skin. As such, each participant is expected to display differing responses: shorter or longer times to bowel evacuation (or the absence of bowel evacuation), as well as the absence or presence of cholinergic (increased bowel activity, bradycardia, bronchoconstriction) or anti-cholinergic activity, as previously described in the text above. The pharmacokinetic data will, to a large extent, reflect this variability in transdermal drug absorption. The data collected will include the presence or absence of bowel evacuation, time to bowel evacuation, consistency (Bristol stool scale), and quantity (by weight). The presence/number of cholinergic and anti-cholinergic side-effects on a standardized point scale will be determined, as well as their severity (e.g., mild, moderate, or severe). Heart rate, blood pressure, oxygen saturation, and pulmonary mechanics (airway patency by impulse oscillation system) will be recorded at baseline and sequentially at intervals the initiation of ION (at 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes).

To quantitate the amount of these agents absorbed into the systemic circulation, pharmacokinetic studies will be performed for 2 hours after the start of transdermal drug delivery. Venous blood (2 ml) will be collected into an EDTA tube for both agents at the following time points: baseline (time zero), 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 360, and 480 minutes. Upon drawing, the blood will be placed in an ice bath and spun using a centrifuge within 5 minutes of collection. Upon completion of 5 minutes of centrifugation, the plasma will be aliquoted into two separate vials, with equal volumes and labeled with date and time of draw, study information, NEO or GLY testing destination, and the subject's unique identifier. The transfer vials will be inserted into dry ice for at least 10 minutes, after which they will be placed into the -80 degrees Celsius freezer. Plasma levels of

NEO and of GLY will be batched and measured at a later date. A file designating the tubes with random numbers associated with the draw times will be created for each subject to conceal the sequence of draw and to attempt the removal of possible bias during the measurement and recording of the concentrations of NEO and GLY. NEO and GLY will be measured by mass spectroscopy (Dr. Qishan Lin; SUNY Downstate Albany Research Laboratory using GE LC-MRM detector).

Trial (2): To determine the reproducibility of the transdermal absorption of NEO and GLY, as well as the potential side-effects, subjects will again receive NEO (0.07 mg/kg) + GLY (0.014 mg/kg) by transdermal administration by use of a wired ION system. Additionally, digital rectal stimulation will be utilized as needed at the discretion of the PI, Dr. Cardozo. Pharmacokinetic data and all other measurements and questionnaires that were previously obtained will be acquired once again and compared to Trial 1.

TABLE OF STUDY EVENTS (Main Study 1)*															
Time Points (in minutes)	BL	0	5	10	15	20	30	45	60	90	120	180	240	360	480
NEO+GLY administration (ION)		x													
Blood collection (2ml per vial)	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Heart rate (HR)	x	x	x	x	x	x	x	x	x	x	x				
Blood pressure (BP)	x	x	x	x	x	x	x	x	x	x	x				
Pulse Oximetry (SPO2)	x	x	x	x	x	x	x	x	x	x	x				
Impulse oscillation (IOS)	x	x	x	x	x	x	x	x	x	x	x				
Assessment of bowel sounds	x	x	x	x	x	x	x	x	x	x	x				
Symptoms Survey	x	x	x	x	x	x	x	x	x	x	x				

*Digital rectal stimulation will be performed at the discretion of the PI, Dr. Cardozo.

Iontophoresis of Neostigmine and Glycopyrrolate* (Study visits 2 & 3)
Drug Concentration
Neostigmine Methylsulfate, USP 5mg/mL solution; 0.07 mg/kg (PCCA, Houston, TX)
Glycopyrrolate, USP 1 mg/mL solution; 0.014 mg/kg (PCCA, Houston, TX)
* These agents will be compounded by the JJPVAMC Research Pharmacy; compounded pursuant to a prescription under FD&C Act Section 503A, following USP <795> for non-sterile compounding. The pharmacy will utilize an electronic weighing scale with a Certificate of Calibration and USP-NF grade powders of NEO and GLY each with a Certificate of Analysis.

The maximum dose of NEO is limited to 10.0 mg and the dose of GLY to 2.0 mg per subject per administration.

Description of the Methods:

Iontophoresis (ION): The ION technology will consist of one Dynatron® iBox™ (Salt Lake City, UT). The iBox will deliver an electric current of 4.0 mA/min x 20 minutes via anode electric patches (this level of current is not appreciated by the subject). A 5 x 5cm (2 x 2 inch) area of skin on the proximal anterior left thigh or upper arm will be cleaned with 70% isopropyl alcohol skin preps, sprayed with a thin layer of 20% Benzocaine (Americaine aerosol spray), and covered with an occlusive plastic film for 15 minutes. The film will then be removed and the skin will be cleansed with de-ionized water followed by alcohol skin prep. Hair removal, using an epilator machine (Remington EP 7030) will be performed by clearing an area of 5 x 5 cm (2 x 2 in) square. The depilated area will be covered with 0.2% of sodium lauryl sulfate (SLS) in de-ionized water solution. NEO and GLY will be deposited into a single patch reservoir, which will then be applied to the prepared skin; the maximum solution with medications applied to the patch will be 4.0 ml. The ground patch will be moistened evenly with 1% citric acid dissolved in 0.9% saline. A 7x7cm skin area under the cathode will be wiped with

alcohol only and the ground electrode will be applied to the skin. The ground electrode's continuous skin contact will be ensured with adhesive tape.

Digital Rectal Stimulation: If any subjects experience hyperactive bowel sounds without a bowel evacuation following the administration of NEO and GLY, digital rectal stimulation will be performed to relax the anal sphincter and induce a bowel evacuation at the discretion of the PI, Dr. Cardozo. Digital rectal stimulation will be performed with the subject lying in a bed on their side. The nurse or physician will insert a lubricated index finger into the rectum in a circular motion for about 30 seconds. This procedure will be repeated every 5 to 10 minutes until the subject's bowel is empty or if no stool comes out after two procedures 5 to 10 minutes apart.

Bowel Sounds: Bowel sounds will be assessed, as described in Table 1, after drug administration for 120 minutes. The abdomen will be auscultated at the right lower quadrant for 30 seconds. The stethoscope will be pressed lightly on the abdomen to listen for bowel sounds, 30 seconds per abdominal quadrant. Two or fewer bowel sounds will be deemed hypoactive and more than 15 bowel sounds in 30 seconds will be deemed hyperactive.

Symptoms Survey: Subjects will be monitored after drug administration for the following symptoms and signs: salivation (increase/decrease), difficulty in swallowing, syncope, blurry vision, eyelid and other muscle fasciculation or cramps, diaphoresis, headache and abdominal discomfort.

Blood Pressure (BP) and Continuous Heart Rate (HR) Monitoring: A baseline EKG will be obtained to exclude participants with EKG abnormalities. The vital signs will be recorded at baseline and then sequentially monitored throughout the experiment using an automated portable blood pressure and heart rate monitor (IVY 101R, IVY Biomedical, Branford, CT) and recorded at intervals after drug administration (at 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes).

Impulse Oscillation System (IOS): Total pulmonary resistances will be measure using a commercially available IOS device (VIASYS Healthcare, Respiratory Technologies, Yorba Linda, CA). Participants will be asked to breath normally (e.g., tidal volume breathing). Resistances will be calculated from the pressure/flow relationship obtained from impulses applied at the mouth during a 30 second period; the data will be analyzed at 5 and 20 Hz (R5 & R20). The data capture for 30 second periods will be repeated until 3 recordings fulfill the quality assurance coherence coefficient criteria (e.g., coherence coefficient criteria of $\geq$0.7 at 5 Hz and $\geq$0.7 at 20 Hz for either 5 breaths or 30 seconds of recording). Large airway mechanics will be inferred responses at high frequencies (20Hz) and low airway mechanics will be inferred responses at low frequencies (20Hz) because low frequency oscillations are transmitted to the lung periphery and those at higher frequencies are limited to the larger caliber airways.

IV Line Insertion: Prior to the transcutaneous administration of study drugs by ION, an intravenous catheter, preferably 20-gauge, will be placed in a peripheral vein in the arm. IV access will be maintained throughout each study visit to allow the administration of a rescue agent in case of an emergency, as well as to serve as a blood draw access point.

Pulse Oximetry: Continuous monitoring of systemic oxygenation will be performed by a hand digit pulse oximeter.

Study 2: **Further Optimizing the Doses of the Drugs for Transcutaneous Administration by ION**
The investigators will have obtained pharmacokinetic data on each participant from Study 1. From the data acquired, the peak plasma concentration (PPC) and the area under the curve (AUC) for NEO will be calculated. Our hypothesis is that the PPC for NEO will correlate more closely with bowel evacuation than its AUC, and

the AUC for GLY will correlate more closely with anti-cholinergic symptoms than its PPC, but this prediction is just our speculation prior to obtaining and analyzing the data. If the plasma concentration of each NEO and GLY are solely responsible for determining biological effects (e.g., cholinergic and anti-cholinergic), then we would predict that there will be a direct and strong correlation of drug levels with those endpoints. However, if the biological effects are primary determined by end-organ responsiveness, then the correlation between drug concentration and observed endpoints will be tenuous, at best. If both plasma concentration and end-organ responsiveness play a role in bowel evacuation and cholinergic side-effect, then an effect of plasma concentrations will still be evident, but not as strong.

Study (2.1): **Determination of a Lower Effective Dose of NEO to Induce Bowel Evacuation**

Because 85-90% of participants are anticipated to have a bowel evacuation from NEO administration at the dose previously employed (standard dose: NEO 0.07 mg/kg), the dose administered is most probably well above the lowest effective dose to achieve the biological desired effect ("ceiling effect"). If the lowest effective dose can be determined, side-effects from NEO administration will be reduced. As such, to identify the upper limit of the lowest effective dose, the dose of NEO will be titrated down until the effect on the primary end organ is lost—that is, until bowel evacuation no longer occurs after drug administration in at least 50% of the participants. Thus, to determine if a lower dose of NEO will still result in bowel evacuation, participants will have the dose of NEO reduced by 25% (with the dose of GLY also reduced by 25%). The total volume of the drugs applied to the patch will be kept constant. In these dose reduction experiments, pharmacokinetic data (e.g., AUC and PPC) will be obtained on each participant for each reduction in dose of NEO. All other measurements and questionnaires that were obtained in Study 1 will be performed.

TABLE OF STUDY EVENTS (Main Study 2)*											
Time Points (in minutes)	BL	0	5	10	15	20	30	45	60	90	120
NEO+GLY administration (ION)		x									
Blood collection (2ml per vial)	x	x	x	x	x	x	x	x	x	x	x
Heart rate	x	x	x	x	x	x	x	x	x	x	x
Blood pressure	x	x	x	x	x	x	x	x	x	x	x
Impulse oscillation (IOS)	x	x	x	x	x	x	x	x	x	x	x
Assessment of bowel sounds	x	x	x	x	x	x	x	x	x	x	x
Symptoms Survey	x	x	x	x	x	x	x	x	x	x	x

*Digital rectal stimulation will be performed at the discretion of the PI, Dr. Cardozo.

<u>Iontophoresis of Neostigmine and Glycopyrrolate* (Study visits 4 & 5)</u>

Drug Concentration

Neostigmine Methylsulfate, USP 5mg/mL solution (PCCA, Houston, TX)

Glycopyrrolate Inj., USP 1 mg/mL solution (PCCA, Houston, TX)

* These agents will be compounded by the JJPVAMC Research Pharmacy; compounded pursuant to a prescription under FD&C Act Section 503A, following USP <795> for non-sterile compounding. The pharmacy will utilize an electronic weighing scale with a Certificate of Calibration and USP-NF grade powders of NEO and GLY each with a Certificate of Analysis.

Dose:

Study visit 4: 0.0525 mg/kg NEO and 0.0105 mg/kg GLY via ION.

Study visit 5: 0.035 mg/kg NEO and 0.007 mg/kg GLY via ION.

(The maximum dose of NEO is limited to 10.0 mg and the dose of GLY to 2.0 mg per subject per administration.)

Possible Risks and Protections Against Risks:

Life threatening Serious Adverse Events: Any study-related events that are life threatening or result in death. Known serious reactions to neostigmine methylsulfate include: respiratory arrest, severe respiratory distress requiring intervention, anaphylaxis with associated hypotension, partial or complete airway obstruction, bradyarrhythmia, or cardiac dysrhythmia accompanied by hemodynamic instability (relative hypotension defined as a drop in MAP >20% post drug administration, or cardiac arrest.

Corrective Action: For all life threatening adverse events that are possibly study-related, the research study will be immediately suspended. All enrolled subjects will be notified about the event, and the appropriate corrective actions taken to ensure that enrolled subjects are not at risk. All local and federal regulatory agencies including the FDA will be notified within 24-hours of the event. At this juncture, a board of clinical experts who are not affiliated with the study will be convened by the principal investigator, including two gastroenterologists, a cardiologist, a biostatistician, a spinal cord injury physician, and a clinical pharmacologist. This board of experts will make a determination as to whether the event was related to the study procedures, and whether the research may continue in light of the available information. The research will be resumed if and only if all regulating bodies [FDA, local IRB and R&D committees, and the assembled board of experts] agree that the event was not related to study procedures. If and when appropriate, alternative procedures or changes to the research design will be implemented so that the risk to benefit ratio remains acceptable.

Serious Adverse Events: Any study related events that require inpatient hospitalization, prolongs hospitalization, results in persistent or significant disability/incapacity, require intervention to prevent permanent impairment or damage, or any other serious medical events. Known serious reactions to neostigmine methylsulfate are convulsions, loss of consciousness, respiratory arrest, atrioventricular block, bradycardia or cardiac dysrhythmia without hemodynamic instability, bronchoconstriction (change in respiratory resistance (Rrs5) >35%, sustained bradycardia (change in heart rate > 30% and/or heart rate is <42 bpm) without relative hypotension (rapid change in MAP of >30% from baseline) requiring resuscitative measures, anaphylaxis, loss of consciousness or seizure.

Corrective Action: For all serious adverse events, all study related activities will be put on hold. All local and federal regulatory agencies will be notified within 72-hours of the event. All enrolled subjects will be notified about the event and the appropriate corrective actions taken to ensure that enrolled subjects are not at risk. Study related activities will not be resumed until all regulating bodies [FDA, local IRB and R&D committees, and the assembled board of clinical experts' agree that study activities can be resumed, or if an adequate plan to change the study is drafted so that the risk-benefit ratio are favorable.

Mild/Moderate Adverse Events: Any untoward medical occurrence in a patient after drug administration; does not necessarily have to have a causal relationship with the treatment. Known common reactions to neostigmine methylsulfate include dizziness, drowsiness, headache, dysarthria, myosis, visual changes, nonspecific EKG changes, relative hypotension (>30% in MAP from baseline) that is not sustained and self-resolving, increased oral, pharyngeal and bronchial secretions, respiratory depression, bronchospasm, rash, urticaria, nausea, emesis, flatulence, increased peristalsis, urinary frequency, muscle cramps and spasms, arthralgia, diaphoresis, flushing and weakness.

Corrective Action: All mild or moderate adverse events will be documented and reported on an annual basis to the local IRB and R&D committees when applying for study continuation. These events will be summarized and submitted as part of the annual progress report to the FDA. All adverse events will be monitored by the study team on an ongoing basis, and by an independent arbitrator who will review the data on a quarterly basis to determine if a pattern of adverse events is emerging. If a pathological finding is discovered through the

review process, all local and federal regulatory agencies will be notified, and study procedures amended to improve the risk to benefit ratio.

Neostigmine Methylsulfate (Adverse reactions may occur if given unopposed by a muscarinic receptor blocker such as Glycopyrrolate)

Adverse events are generally due to an exaggeration of pharmacological effects of which salivation and fasciculation are the most common. Bowel cramps and diarrhea may also occur.

The following additional adverse reactions have been reported following the use of Neostigmine Methylsulfate: allergic reactions and anaphylaxis, dizziness, convulsions, loss of consciousness, drowsiness, headache, dysarthria, miosis, visual changes, cardiac arrhythmias (including bradycardia, tachycardia, A-V block and nodal rhythm), nonspecific EKG changes, cardiac arrest, syncope, hypotension, increased oral, pharyngeal and bronchial secretions, dyspnea, respiratory depression, respiratory arrest, bronchospasm, urticaria, nausea, emesis, flatulence, increased peristalsis, urinary frequency, muscle cramps, arthralgia, diaphoresis, flushing and weakness.

Overdose of Neostigmine Methylsulfate can cause cholinergic crisis, which is characterized by increasing muscle weakness, and through the involvement of the muscles of respiration, may result in death. Myasthenic crisis, due to an increase in the severity of the disease, is also accompanied by extreme muscle weakness and may be difficult to distinguish from cholinergic crisis on a symptomatic basis. However, such differentiation is extremely important because increases in the dose of Neostigmine Methylsulfate or other drugs in this class, in the presence of cholinergic crisis or of a refractory or "insensitive" state, could have grave consequences. The two types of crises may be differentiated by the use of edrophonium chloride as well as by clinical judgement. Treatment of the two conditions differs radically. Whereas the presence of myasthenic crisis requires more intensive anticholinesterase therapy, cholinergic crisis calls for the prompt withdrawal of all drugs of this type. The immediate use of Atropine in cholinergic crisis is also recommended. Atropine may also be used to abolish or minimize gastrointestinal adverse events or other muscarinic reactions; but such use, by masking signs of overdosage, can lead to inadvertent induction of cholinergic crisis. The LD50 of Neostigmine Methylsulfate in mice is 0.3 ± 0.02 mg/kg intravenously, 0.54 ± 0.03 mg/kg subcutaneously and 0.395 ± 0.025 mg/kg intramuscularly; in rats the LD50 is 0.315 ± 0.019 mg/kg intravenously, 0.445 ± 0.032 mg/kg subcutaneously and 0.423 ± 0.032 mg/kg intramuscularly.

Glycopyrrolate (Adverse reactions if not administered in opposition to a cholinesterase inhibitor such as Neostigmine)

Anticholinergics, including Glycopyrrolate Injection, can produce certain effects, most of which are extensions of their pharmacologic actions. Adverse reactions may include xerostomia (dry mouth); urinary hesitancy and retention; blurred vision and photophobia due to mydriasis (dilation of the pupil); cycloplegia; increased ocular tension; tachycardia; palpitation; decreased sweating; loss of taste; headache; nervousness; drowsiness; weakness; dizziness; insomnia; nausea; vomiting; impotence; suppression of lactation; constipation; bloated feeling; severe allergic reactions including anaphylactic/anaphylactoid reactions; hypersensitivity; urticaria, pruritus, dry skin, and other dermal manifestations; some degree of mental confusion and/or excitement, especially in elderly persons.

Neostigmine given IV 2 minutes prior to Glycopyrrolate:

Post-marketing reports have included cases of heart block and QTc interval prolongation associated with the combined use of glycopyrrolate and an anticholinesterase (Neostigmine in this case). Giving the NEO a minute or two earlier than GLY diminishes the severity of the GLY adverse events.

Data, Safety and Monitoring Plans:

The maximum dose of NEO is limited to 10.0 mg and the dose of GLY to 2.0 mg per subject per administration. Subjects will be asked to arrive at the Spinal Cord Research Center at the JJP VAMC (Room 7A-13) on the day of their appointment. A baseline EKG assessment will be conducted and analyzed for bradycardia and rhythm abnormalities. The ECG will be read in real time by study investigator to ensure patient safety. Subjects who are found to have old or post-medication administration, new-onset ECG abnormalities, will not receive treatment and will be monitored and followed to resolution of the new ECG abnormalities. Blood pressure, heart rate, cholinergic and anticholinergic symptoms, and bowel sounds will be recorded at baseline. After establishing an IV access point, administration of the medications via IV will be performed on day 1 only. An IV access point will be established and maintained on days 2-5 of the study for blood collection and to allow the administration of a rescue agent in case of an emergency. Blood pressure, heart rate, cholinergic and anticholinergic symptoms, and bowel sounds will be recorded at regular intervals for at least 2 hours (120 minutes) and until all values return to baseline reading levels for all 5 days of the study. Resuscitation equipment and antidote medications will be in close proximity or inside of the room of the experiment. IV reversal Atropine for NEO cholinergic effects at (0.5mg via IV for post-IV GLY and 0.3mg IM for post-ION GLY) and Physostigmine at (1mg via IV for post-IV NEO and 0.5mg IM for post-ION NEO) for reversal of glycopyrrolate anticholinergic effects will be available during all experiments.

A member of the study team will be present at all times with the subject and a physician will be in close proximity. Subjects will be monitored continuously throughout the study visits. All subjects enrolled will be under the direct care and supervision of the principal investigator, the study coordinator, and the study physician. The study investigators will meet on a monthly basis to review the data and adverse events for any identifiable trends. All AEs and SAE's will be reported without exception to the local IRB. Serious adverse events will be reported immediately. If any significant trend is detected, subject recruitment and testing will be discontinued for project evaluation and modified as necessary

To ensure confidentiality, data and the subjects' records will be stored on a VA server located at the James J. Peters VAMC which is behind a VA firewall and not on any individual computer hard drive. Access to this computer storage system is password protected with access to shared project data files limited to individually-authorized project staff members. Further, remote access is, and will continue to be, limited to authorized users of the VA Virtual Private Network (VPN) controlled by the NSOC and authenticated by the JJP VAMC Bronx Information Security Officer in compliance with VA policy (VA Directive and Handbook 6500). The database for computation and analysis will be stored on this VA server so that these raw data files will remain unchanged if there are computational errors or computer problems. Once this study has been completed, the de-identified research records will be retained in accordance with the record control schedule. Access to specific data files will be protected by strong passwords (numbers, special symbols, upper and lower case letters), provided only to project staff members authorized to access to the data. In addition, incremental back-ups of data on servers will be performed weekly with full back ups completed on a monthly basis. Back up media will be removed and stored in a physically secure location within the Center of Excellence (JJP VAMC). External access to systems via an enterprise gateway already is and will continue to be strictly controlled and monitored by the VA Network Security Operations Center (NSOC). Hard copies of subjects' intake and raw data will be stored in locked files in the investigator's VAMC Office in room 7A-13.

Stopping Criteria:

- Burns of the skin as evidenced by redness or swelling that persists for longer than 8 hours, blistering, discoloration or changes in the consistency of the skin.
- Pain at the site of the patches either at time of delivery or after
- Vomiting
- Moderate to severe headache

- Spike in systolic blood pressure to a level of at least 160mm Hg, or an increase of greater than 40mm Hg.

Statistical Analyses:

Specific Aim 1: To determine a lower effective dose of NEO to induce bowel evacuation by transcutaneous administration by ION.

A series of bivariate tests will be carried out for each of the outcome variables of interest (e.g., presence or absence of evaluation, time to evacuation, etc.) and presence/number of cholinergic and anti-cholinergic side effects for each dose level as they relate to PCC and AUC.[12,13] Since the distribution of some of the outcome variables (e.g., time to evacuation) is believed to be non-normal, non-parametric statistical procedures (e.g., Kendall's tau) will be used. From these analyses, we anticipate identifying likely dose-response profiles which can then be tested in future research.

Limitations of the Study:

Pitfall 1: Relatively large individual variability may exist in the relative absorption of NEO and/or GLY by transdermal administration. This would be an important observation, and one that can be made only after generating the pharmacokinetic data. However, this is somewhat unlikely because we have observed effective bowel evacuation with only mild anti-cholinergic side effects in several subjects in our studies to date.

Pitfall 2: Individual variability in drug responsiveness may be larger than anticipated. Again, this can only be determined after generating the pharmacokinetic data (i.e., PPC and AUC to bowel evacuation) and comparing it to the biological responses (bowel evacuation and side-effects), and this would be a highly relevant finding.

Pitfall 3: While the small sample sizes proposed in our study are typical for initial dose determination and device testing, the small samples may not provide sufficient evidence for the use of NEO and GLY to safely and effectively produce bowel evacuation in the general SCI population. Prior data published from our group over the past decade has demonstrated that this drug formulation is effective in stimulating bowel evacuation in a large majority of persons with SCI with tolerable, and no major, side effects. A larger clinical trial should be considered in subjects with SCI that applies the drug formulation determined to be optimal in the current study using ION systems that are wired.

There are a few limitations to the proposed study. There has been no work to date on the pharmacokinetics of either transcutaneous administration of NEO or GLY by ION. As such, much of the studies proposed are from general knowledge of drug absorption, drug elimination, and the relationship of PPC and AUC to the desired biological effect and the adverse effects. Although our preliminary data has suggested (1) 75% of participants will have a response to IV NEO and (2) 85-90% of participants will have bowel evacuation to standard dose of transdermal NEO, this may not be the case. However, the enrollment may be flexibly adjusted to meet the needs of the study. Whatever the results generated from the proposed work, new and relevant knowledge of this mode of administration of this drug combination will be attained. One key component to bringing NEO + GLY to the clinical realm remains the collective objective of this application–that is, obtaining the pharmacokinetics of this drug combination and defining a lower effective dose of NEO to prevent or reduce side-effects.

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