Programmable Transdermal Clonidine Delivery Through Voltage-Gated Carbon Nanotube Membranes

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ABSTRACT: Oral dosage forms and traditional transdermal patches are inadequate for complex clonidine therapy dosing schemes, because of the variable dose/flux requirement for the treatment of opioid withdrawal symptoms. The purpose of this study was to evaluate the *in vitro* transdermal flux changes of clonidine in response to alterations in carbon nanotube (CNT) delivery rates by applying various electrical bias. Additional skin diffusion studies were carried out to demonstrate the therapeutic feasibility of the system. This study demonstrated that application of a small electrical bias (−600 mV) to the CNT membrane on the skin resulted in a 4.7-fold increase in clonidine flux as compared with no bias (0 mV) application. The high and low clonidine flux values were very close to the desired variable flux of clonidine for the treatment of opioid withdrawal symptoms. Therapeutic feasibility studies demonstrated that CNT membrane served as the rate-limiting step to clonidine diffusion and lag and transition times were suitable for the clonidine therapy. Skin elimination studies revealed that clonidine depletion from the skin would not negatively affect clonidine therapy. Overall, this study showed that clonidine administration difficulties associated with the treatment of opiate withdrawal symptoms can be reduced with the programmable CNT membrane transdermal system. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1829–1838, 2014

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INTRODUCTION

Opioids induce a euphoric state or relieve distress with chronic exposure, resulting in adaptive changes in the central nervous system leading to dependence and addiction.[1] Heroin is the most widely abused and fastest acting of the opioid family. In fact, nearly 3.7 million Americans report having used heroin in their lifetime, with over 281,000 treated in clinics annually for opioid addiction.[2] Unfortunately, only 50% of treatment-seeking individuals complete the clinic stay and detoxification.[3] Lack of compliance in the first couple days of treatment can be attributed to the harsh withdrawal effects a heroin-dependent person experiences, including lacrimation, rhinorrhea, piloerection, gastrointestinal cramping, nausea, vomiting, yawning, sneezing, muscle pain, tachycardia, and hypertension.[4] Clonidine has been shown to mitigate these noradrenergic symptoms and thereby aid in opioid abstinence.[5–8] Unfortunately, dosing schemes are complex and require multiple oral doses throughout the clinic stay.[3] Transdermal clonidine systems are commercially available and have been applied to heroin withdrawal treatment.[9–14] Although currently available transdermal clonidine delivers the drug at a constant rate over a week[15–18] and avoids some of the problems associated with oral dosing, such as gastrointestinal complications and subsequent inadequate dosing, the required variable plasma concentrations of drug during the week-long withdrawal treatment period are currently complicated when using traditional transdermal patches There is a need for a single transdermal patch that can have variable clonidine delivery rates in a compact programmable device.

A new class of conductive permeable membranes based on carbon nanotubes (CNTs) has recently been developed[19,20] with dramatic flow properties[21] that can result in very power-efficient pumping with chemical modification.[22] A fourfold reduction of flux of a large dye molecule through CNT membranes by the application of an electrical bias has been previously demonstrated by Majumder et al.[23] However, more recently, it was found that the phenomena of electroosmosis and electrophoresis can pump both neutral and charged drugs at 20-fold flux enhancement compared with diffusion with a 40-fold reduction in power compared with conventional membrane materials. This increase in power efficiency would allow a standard watch battery to continuously transdermally deliver high doses of nicotine for 12 days.[24] In the case of opioid withdrawal treatment, these systems could be programmed and tailored to a specific individual's needs, and furthermore eliminate the difficulties of multidose administration associated with patient compliance. Pulsatile delivery has been shown to be achieved across the skin using methods to enhance permeability, such as iontophoresis[25]; however, the CNT membrane transdermal

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patch is novel in that it does not use skin enhancement methods with irritation side effects and high power requirements. In this case, drugs are to be delivered at a variable rate to the stratum corneum.

This study shows that the incorporation of programmable CNT membranes with a transdermal formulation of clonidine was successful in varying delivery rates of a known variable clonidine treatment regimen for opioid withdrawal. This affords the opportunity to manipulate the flux, and thereby dose of a specific drug to a patient during their rehab stay or in outpatient settings of relapse with counseling. Utilizing programmable membranes such as these in the drug delivery field would open an avenue for highly effective, one time application patches for therapies that require variable rate delivery and the potential for remote programming in outpatient relapse care with associated telephone or internet counseling. In this study, it was important to demonstrate the therapeutic feasibility of delivering successive variable rates of clonidine through human skin. Additional studies of gel formulation and polymer/adhesive permeation were also conducted for future prototype development goals. The first aim of the present study was to investigate clonidine flux through the skin, and to assess whether skin responds to successive changes in the flux without excessive lag times or depot effects. The second aim was to focus on the drug permeation from solution versus gel formulations, of which the latter is required for reservoir patch drug systems. The third aim was an investigation of high permeability polymer membrane and pressure-sensitive adhesive (PSA) systems, which will be required for a commercial prototype patch utilizing the CNT membrane. The fourth aim was to investigate the elimination characteristics of clonidine from the skin as this depot of drug will still be delivered to systemic circulation upon deactivation or complete removal of the patch. The fifth and final aim of this study was to show controllable delivery of the drug through the CNT membrane alone and through the CNT membrane/skin system *in vitro* as a function of applied bias.

MATERIALS AND METHODS

Materials

Clonidine hydrochloric acid (HCl) and acetonitrile (ACN) were purchased from VWR International, LLC (West Chester, Pennsylvania). Clonidine HCl was converted to clonidine base. Potassium phosphate monobasic, dibasic potassium phosphate, sodium chloride, ammonium bicarbonate, ammonium hydroxide, and HCl were purchased from Fisher Scientific Inc. (Fair Lawn, New Jersey). Hydroxyethylcellulose (HEC; Natrosol®) was used as the gelling agent for donor solutions as well as for contact gel between the skin and CNT membrane and was obtained from Hercules Inc. (Wilmington, Delaware). Deionized distilled water was generated from the Barnstead Nanopure Diamond™ system (Dubuque, Iowa).

Fabrication and Functionalization of CNT Membranes

Multiwalled carbon nanotubes (MWCNTs) with an average core diameter of approximately 7 nm were fabricated on quartz substrate via a chemical vapor deposition approach using ferrocene/xylene as the feeding gas.[19] The length of MWCNTs ranges from 100 to 150 μm as measured by DekTak Profilometer. In the next step, MWCNTs were mixed thoroughly with Epon 862 epoxy resin (Miller Stephenson Chemical Company, Danbury, Connecticut), surfactant Triton-X 100 (Sigma–Aldrich, St. Louis, Missouri), catalyst 1-cyanoethyl-2-ethyl-4-methylimidazole (2E4MZ-CN; Shikoku Chemical, Tokyo, Japan), and hardener methylhexahydrophthalic anhydride (Broadview Technologies Inc., Newark, New Jersey) using a ThinkyTM Mixer. The appropriately cured CNTs–Epoxy composite was cut into approximately 5 μm thick membranes using a microtome equipped with a glass knife. The residual epoxy on the tips of CNT was removed via a following H_2O plasma oxidation. Double-walled CNT (~2 nm core diameter; Cheaptubes Inc., Brattleboro, Vermont) membranes were prepared using a similar method as described above. Porosities of both MWCNT and DWCNT membranes were screened by ion diffusion flux. Individual membrane samples were chosen with highest porosity (~0.01%) and within 20% variation. The as-cut CNT membranes were functionalized using a two-step process. In the first step, CNTs were grafted with benzoic acid through electrochemically reducing 5 mM 4-carboxy phenyl diazonium tetrafluoroborate in 0.1 M HCl and 0.1 M KCl electrolyte at −0.6 V for 4 min.[23] The diazonium compound was synthesized following a method reported by D'Amour and Bélanger.[26] The benzoic acid was then coupled to Direct Blue 71 dye via a carbodiimide coupling reaction. Specifically, 10 mg 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (98%; Aldrich, St. Louis, Missouri) was added to 4 mL of 50 mM Direct Blue 71 dye in 0.1 M 2-N-morphilino ethanesulfonic acid (MES, 99%; Sigma, St. Louis, Missouri) buffer, and the reaction lasted for 12 h at ambient temperature, after which the membrane was rinsed thoroughly using 0.1 M MES buffer, 0.1 M KCl solution, and deionized water to remove unreacted chemicals.

Human Skin Preparation

Human skin harvested during abdominoplasty was used for the transdermal clonidine delivery studies and obtained from the Cooperative Human Tissue Network (CHTN). Human tissue use was approved by the University of Kentucky Institutional Review Board. Skin sections were prepared using a Padgett dermatome set to 250 μm and then stored at −20°C until use. Stored skin samples were thawed to room temperature at the time of the experiment.

Clonidine Skin Studies

A PermeGear flow-through (In-Line, Hellertown, Pennsylvania) diffusion cell system with a diffusion area of 0.07 cm^2 was used for all *in vitro* skin diffusion studies. Skin surface was maintained at 32°C with a circulating water bath. Stratum corneum integrity was checked before all experiments using TEWL instrumentation (RG1 Evaporimeter; cyberDERM Inc., Broomall, Pennsylvania). Donor solutions for all skin diffusion studies were clonidine in water. Donor concentrations were varied based on the study being conducted and are mentioned in the respective sections. The receiver solution was 0.9% saline with 10% ethanol and flowed at a 7 mL/h rate. Samples were collected every 3 h, and the diffusion study was conducted for 24 h unless otherwise stated in the sections below. All samples collected were stored at 4°C until analyzed by HPLC.

Determination of Maximum Flux

Dermatomed human abdominal skin sections were loaded into the diffusion cells, and varying concentrations of clonidine solution (325 and 950 μg/mL) were applied directly to the skin.

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