Published in final edited form as:

Cardiovasc Ther. 2017 August ; 35(4): . doi:10.1111/1755-5922.12269.

The use of an occlusion perfusion catheter to deliver paclitaxel to the arterial wall

Marzieh K. Atigh¹, Emily Turner¹, Uwe Christians, Ph.D.², and Saami K. Yazdani¹

¹Mechanical Engineering Department, University of South Alabama, Mobile, AL, 36688, USA

²iC42 Clinical Research and Development, University of Colorado, Aurora, CO 80045, USA

Abstract

Aim—Non-stent drug delivery platforms have recently emerged as an alternative treatment of peripheral arterial disease. Perfusion catheters have the potential to directly deliver anti-proliferative agents to the medial arterial layer to prevent restenosis. The purpose of this study was to therefore determine the effectiveness of a perfusion catheter to deliver paclitaxel, a proven anti-proliferative agent, to combat restenosis.

Methods—A bench-top model was utilized to determine the varying parameters of a novel occlusion perfusion catheter to maximize paclitaxel delivery using pharmacokinetic evaluation and fluorescent microscopy. Parameters tested included concentration of paclitaxel, delivery pressure, duration of delivery, and the use of an excipient. In addition, bilateral rabbit iliac arteries were treated with the perfusion catheter and pharmacokinetic evaluation performed at 1 hour, 1 day and 3 days.

Results—Bench-top testing demonstrated uniform and circumferential penetration of paclitaxel within the treated arteries. The results of the ex vivo test identified two groups with and without an excipient with similar loading conditions (with excipient: 15.4 ± 8.6 ng/mg vs. without excipient: 8.9 ± 6.9 ng/mg, $p = 0.77$). The in vivo pharmacokinetic analysis of these two groups demonstrated the use of contrast agent increased arterial paclitaxel levels and maintained initial paclitaxel dosing up to 3 days (With excipient: 1 hr: 107 ± 62 ng vs. 3 days: 40 ± 23 ng, $p = 0.824$; No excipient: 1 hr: 247 ± 120 ng vs. 3 days: 2.92 ± 2.9 ng, $p = 0.009$).

Conclusions—These results demonstrate the feasibility to deliver paclitaxel directly to the medial layer of an artery via a perfusion catheter.

Correspondence: Saami K. Yazdani, PhD, Assistant Professor, Department of Mechanical Engineering, University of South Alabama, Mobile, AL 36688, Phone (251) 460-7220, Fax (251) 460-6549, syazdani@southalabama.edu.

Author contributions: Marzieh K. Atigh – Data collection, critical revision of article, approval of article

Emily Turner – Data collection, critical revision of article, approval of article

Uwe Christians – Data analysis, critical revision of article, approval of article

Saami K. Yazdani - Data collection, statistic, data analysis, critical revision of article, approval of article, securing funding,

Disclosures: Saami K. Yazdani serves on the Scientific Advisory Board of Advanced Catheter Therapies and Toray Industries and has received grant support from Advanced Catheter Therapies and Lutonix, Inc., and is a shareholder in Advanced Catheter Therapies.

Other co-authors have no conflict of interest to report.

Keywords

interventional cardiology; perfusion catheter; paclitaxel; animal model; pharmacokinetics; local drug delivery

Introduction

The treatment of peripheral arterial disease (PAD) costs the US roughly \$21 billion annually and affects more than five million people. Despite this sizeable economic investment, treatment outcomes remain poor. Percutaneous intervention, balloon angioplasty and stenting, are the preferred choice to treat PAD due to reduced morbidity, mortality and in-hospital stay as compared to bypass surgery^{1,2}. However, results are sub-optimal with high rates of restenosis³, re-occlusion of the treated artery. Historically, stent fractures, either drug coated or bare, are a big contributor to this poor performance with reported restenosis rates up to 76% in fractured stents^{4,5}. More recent data demonstrates superficial femoral stent fracture rates of 12.5% to 30%, with associated restenosis rates of 10% to 56.6%^{6,7}. The use of stents for below-the-knee (BTK) applications is also limited due to long lesion length (130 to 170 mm) of BTK lesions, requiring multiple stents. BTK stent implantations are thus primarily used as bailout strategy in case of flow limiting dissection or recoil⁸. Treatment of BTK vessels is essential for limb salvage in diabetic patients with PAD^{9,10}. In these instances, balloon angioplasty is the only option, however, restenosis rates of balloon angioplasty are inferior to stents¹¹⁻¹⁹.

The recently approved drug coated balloon (DCB) has emerged as an alternative strategy to treat femoropopliteal segments²⁰. DCB deliver anti-proliferative agents, such as paclitaxel, in combination with excipients (drug carriers) to the arterial wall^{21,22}. Excipients thus play an essential role in the delivery and retention of paclitaxel in non-stent drug delivery platforms. The fundamental approach of DCB, however, is similar to drug eluting stents (DES) in that the anti-proliferative drugs are stored on the intima, leading to potential problems in delayed healing, poor endothelialization, and the potential for thrombosis²³. Furthermore, coating design and choice of excipient of DES in addition to the wide range of biomechanical movement in peripheral arteries²⁴ has the potential to dislodge the drug/excipient at the luminal surface (emboli) and occlude smaller arteries downstream. This may be particularly detrimental for a diabetic patient with very poor foot circulation²⁵⁻²⁷. Both DES and DCB are also limited as they are designed as a single-use device, delivering anti-proliferative drugs only to one location.

The Occlusion Perfusion Catheter™ (OPC™, Advanced Catheter Therapies, Chattanooga, TN) is a novel multi-lumen catheter designed to occlude the target lesion from blood flow, flush the blood from the treatment chamber, and then locally deliver the therapeutic agents into the vessel. The delivery of therapeutic agent is then accomplished by pressure differences created by an increase in luminal pressure by the perfusion catheter thereby driving the drug molecules across tissue layers into the medial wall. The potential benefit of this approach is that it avoids the accumulation of anti-proliferative drugs on the luminal

surface, as the drug is delivered into the medial wall. The design of the perfusion catheter also permits treatment of multiple sites with a single device in the same patient.

The purpose of this study was to therefore determine the effectiveness of the OPCTM perfusion catheter to deliver paclitaxel, a proven clinical anti-restenotic agent²⁸. Specifically, parameters impacting the delivery and retention of paclitaxel were investigated using bench top and a clinically relevant pre-clinical model.

Methods

Bench-Top Bioreactor System

Twenty-eight porcine carotid arteries were harvested from large pigs (110-160 kg) from a local abattoir (Semmes, AL) and transferred in sterile PBS with 1% antibiotics-antimicrobials (Gibco, Grand Island, NY, 14072). Vessels were then rinsed in sterile PBS in a culture hood. The excess fat, connective tissue, and fascia were removed from each vessel. Vessels were cut into approximately 8 cm segments and stored in 15-mL centrifuge tubes at -20°C until needed. Frozen vessels were thawed in a 37°C water bath and placed into the bioreactor system to be studied.

The bioreactor system used in this study consisted of a flow reservoir, pump, vessel housing compartment, and a distal flow constrictor (Fig. 1). The pressure was monitored via a catheter pressure transducer (Millar Instruments, Houston, TX). The flow was monitored by an ultrasonic flow meter (emtec flow technology, Berkshire, NY) that was positioned prior to the bioreactor. A custom LabVIEW program was used to generate pulsatile waveforms and to control and monitor the mechanical conditions (flow and pressure) within the bioreactor system. The bioreactor medium consisted of Dulbecco's modified eagle's medium (DMEM) containing low glucose, [1000 mg/L], 4.0 mM L-glutamine, 110 mg/L sodium pyruvate, pyridoxine hydrochloride, 10% fetal bovine serum (Gibco), and 1% antibiotic-antimycotic (Gibco).

Drug Delivery via Perfusion catheter

To deliver paclitaxel locally to selected regions, a multi-lumen balloon perfusion catheter (Advanced Catheter Therapies, Chattanooga, TN) was utilized. Prior to paclitaxel delivery, ex vivo arteries underwent endothelial denudation using an angioplasty balloon catheter (5.0 mm × 15 mm). The perfusion catheter can temporarily occlude the target area from blood flow by deploying occlusion balloons (Fig. 2). The treatment chamber can then be flushed to remove trapped blood. Following flushing of the treatment chamber, therapeutic agents/drugs can then be delivered to the treatment chamber. The delivery of therapeutic agent is accomplished by pressure differences created by an increase in luminal pressure by the perfusion catheter thereby driving the drug molecules across tissue layers into the medial wall. Paclitaxel (6.0 mg/ml, Sagent Pharmaceuticals, Schaumburg, IL) was delivered to each harvested artery at varying concentrations ranging from 1.2 mg/ml to 6.0 mg/ml. The 6.0 mg/ml paclitaxel concentration was the maximum (stock) solution of the liquid paclitaxel, and the 1.2 mg/ml was the manufacturer recommended dilution of paclitaxel, and 3.6 mg/ml was the median value. Lower concentrations (<6.0 mg/ml) were achieved by diluting

paclitaxel with saline and iohexol (Ominipaque™, GE Healthcare, Wauwatosa, WI), which served as an excipient. Specifically, to dilute the solution to lower concentrations, the 6 mg/ml was diluted in saline at a ratio of 1:4 (1-part paclitaxel, 4-part saline) to achieve a 1.2 mg/ml and 3:2 (3-part paclitaxel, 2-part saline) to achieve a 3.6 mg/ml paclitaxel concentration. For the excipient portion, the solution was diluted at a ratio of 3:1:1 (3 parts paclitaxel, 1-part saline, 1-part iohexol).

Rabbit injury model

This study was approved by the Institutional Animal Care and Use Committee and conformed to the position of the American Heart Association on use of animals in research. The experimental preparation of the animal model has been previously reported²⁹. Under fluoroscopic guidance, nine anesthetized adult male New Zealand White rabbits underwent endothelial denudation of both iliac arteries using an angioplasty balloon catheter (3.0 mm × 8 mm). Subsequently, paclitaxel was delivered via the perfusion catheter (3.0 mm × 15 mm) at a delivery pressure of 2 atm for two minutes. The treatment chamber volume for the perfusion catheter was 0.0744 ml, delivering 89 µg (0.84 µg/mm³) for the 1.2 mg/ml group and 268 µg (2.53 µg/mm³) for the 3.6 mg/ml group. Anti-platelet therapy consisted of aspirin (40 mg/day) given orally 24 hours before catheterization with continued dosing throughout the in-life-phase of the study, while single dose intra-arterial heparin (150 IU/kg) and lidocaine was administered at the time of catheterization.

Following 1 hour, 1 day and 3 days, animals were anesthetized, euthanized (intravenous fetal plus, 85-150 mg/kg, single injection), and the treated segments removed based on landmarks identified by angiography. These time points were selected to demonstrate the acute retention of paclitaxel following delivery. The arteries were then stored at -80°C and shipped on dry ice to the bioanalytical laboratory (iC42 Clinical Research and Development, Aurora, CO).

Arterial Sections

To demonstrate drug penetration by the perfusion device, *ex vivo* arterial segments were cut from the arterial test section and frozen fresh in optical cutting temperature (O.C.T.) compound (Sakura Finetek USA, Inc., Torrance, CA). Arterial cross-sections were cut at 10 micron thickness and imaged with a Nikon Eclipse TI confocal microscope (Nikon Instruments Inc., Melville, NY). Fluorescently labeled paclitaxel, Flutax-1 (Tocris Bioscience, Bristol, UK) was excited at 488nm and detected at 525/50 nm.

Quantification of Paclitaxel Tissue Concentrations

For quantification of paclitaxel, a previously described, validated high-performance liquid chromatography (HPLC)-electrospray ionization- tandem mass spectrometry system (LC-MS/MS) was used³⁰. The LC-MS/MS system consisted of a series 1260 HPLC system (Agilent Technologies, Santa Clara, CA) and an ABSciex 5000 triple-stage quadrupole mass spectrometer (ABSciex, Concord, ON). Briefly, Paclitaxel and the internal standard Paclitaxel-D₅ was purchased from Toronto Research Chemicals (Toronto, ON). Tissue calibration curves were prepared using homogenized untreated pig artery tissues and had the following concentrations: 0, 0.5, 1, 2.5, 5, 10, 25, 50, 100 ng/mL; and the quality control

samples run during study sample analysis had the following concentrations: 0.75, 7.5 and 75 ng/mL. One mL of phosphate buffer was added to the tissue samples. The samples were then homogenized using an electric wand homogenizer (VWR 200, VWR International, Radnor, PA). Two hundred μ L of the diluted sample, calibrator, and quality control samples were aliquoted and 800 μ L of 0.2 M ZnSO_4 30% water/ 70% methanol v/v containing the internal standard (10 ng/mL) was added. Samples were vortexed for 5 min, centrifuged (16,000, 4°C, 15 min) and transferred into HPLC vials. Study samples were diluted as necessary for detector signals to fall within the reliable range of quantification of the assay.

One hundred μ L of the samples was injected onto a 4.6 $\times$ 12.5 mm 5 μ m extraction column (Eclipse XDB C-8, Agilent Technologies, Palo Alto, CA). Samples were washed with a mobile phase of 15% methanol and 85% 0.1% formic acid. The flow was 3 mL/min and the temperature for the extraction column was set to 65 °C. After 1 min, the switching valve was activated and the analytes were eluted in the backflush mode from the extraction column onto a 150 $\times$ 4.6 mm C8, 3.5 μ m analytical column (Zorbax XDB C8, Agilent Technologies, Palo Alto, CA). The mobile phase consisted of methanol/acetonitrile (1/1 v/v) plus 0.1% formic acid (solvent B) and 0.1% formic acid in HPLC grade water (solvent A). The following gradient was run: 0 to 1 minutes: 60% solvent B, 1 to 2 minutes: 60% to 98% solvent B, 2 to 4 minutes: held at 98% solvent B, 4 to 4.1 minutes: 98% to 60% solvent B, and stayed at 60% acetonitrile/methanol plus 0.1% formic acid for the next 0.5 min. The MS/MS was run in the positive multi-reaction mode. For Paclitaxel, the following ion pair was detected: $m/z = 876.6 [M+Na]^+ \rightarrow 308.2$. Paclitaxel-D₅, the internal standard, was detected using the transition $m/z = 881.6 [M+Na]^+ \rightarrow 313.1$. Paclitaxel tissue concentrations were calculated based on the peak area ratios of Paclitaxel and its internal standard. A quadratic regression equation with 1/x weighting was used. The range of reliable response was 0.5- 100 ng/mL tissue homogenate. Inter-day imprecision was less than 15% and accuracy was within 85-115% of the nominal concentrations. There were no significant matrix interferences, carry-over or matrix effects.

Statistical Analysis

All values were expressed as mean $\pm$ standard deviation (SD). Quantitative data were compared with analysis of variance (ANOVA) using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). Comparison of multiple groups was performed by Tukey's multiple comparisons *post hoc* test. A value of $p < 0.05$ was considered statistically significant.

Results

Bench Top modeling

Figure 3 shows a representative image of a porcine carotid artery being treated by a perfusion catheter within the ex vivo bioreactor system. The explanted vessels were pulsed for 1 hour, where they were subjected to physiological flow conditions and varying paclitaxel concentrations (Figure 1, Table 1). Arterial paclitaxel levels increased as the delivered paclitaxel concentration increased from 1.2 mg/ml to 6.0 mg/ml, although there was a dip at 3.6 mg/ml (8.9 ± 6.9 ng/mg vs. 5.1 ± 3.9 ng/mg vs. 21.2 ± 10.4 ng/mg; $p = 0.035$). This pattern is more likely due to high variability of pharmacokinetic data at acute time

points³¹. The addition of excipient also increased arterial paclitaxel levels at 1 hour for both the 1.2 mg/ml (8.9 ± 6.9 ng/mg vs. 13.2 ± 9.5 ng/mg; $p = 0.49$) and 3.6 mg/ml (5.1 ± 3.9 ng/mg vs. 15.4 ± 8.6 ng/mg; $p = 0.197$), although differences were not significant. Increase in delivery duration from 2 to 4 minutes (15.4 ± 8.6 ng/mg vs. 20.7 ± 4.70 ng/mg; $p = 0.49$) and an increase in delivery pressure from 2 to 4 atm (15.4 ± 8.6 ng/mg vs. 25.9 ± 11.28 ng/mg; $p = 0.28$) had no significant impact on arterial paclitaxel concentration.

To visualize drug penetration, fluorescent paclitaxel (Flutax-1, 0.1 mg/ml) was delivered with and without excipient. Representative images using confocal microscopy demonstrated uniform circumferential delivery of the Flutax-1 using the OPC perfusion catheter, although less penetration into the medial layer was observed with the excipient group (Fig. 3).

Pre-clinical animal modeling

Following ex vivo studies, parallel in vivo studies were performed using the rabbit ilio-femoral injury model to determine the impact of excipient on arterial paclitaxel retention. Arteries were treated with either paclitaxel alone (Group I) or paclitaxel with an excipient (Group IV) using the OPC perfusion catheter. An angiogram of the perfusion catheter within the ilio-femoral artery is shown in Figure 4. Table 2 summarizes the arterial concentration (ng/mg) results of the rabbit study. No significant differences were observed between any time points for the paclitaxel concentration measurements. Paclitaxel loading (total paclitaxel retained, ng/mg multiplied the weight of tissue measured, Fig. 4) results demonstrated a significant decrease at 1 hour to 3 days in the non-excipient group (1 hr: 246.9 ± 120.3 ng vs. 3 days: 2.92 ± 2.91 ng, $p = 0.009$), whereas no significant decrease was observed with the excipient group (1 hr: 107.7 ± 62.1 ng vs. 3 days: 40 ± 23.0 ng, $p = 0.824$).

Discussion

This study was designed to evaluate, for the first time, the drug delivery of a novel occlusion perfusion catheter and the impact of varying parameters (delivery pressure, drug concentration, duration time) and excipients on the uptake of paclitaxel using an ex vivo and in vivo rabbit ilio-femoral injury model. Both models utilized healthy vessels without the presence of side branches or bifurcations. Confocal microscopy analysis of perfusion catheter-treated explanted swine carotid arteries demonstrated uniform and circumferential penetration of fluorescently labeled paclitaxel (Flutax-1) within the treated arteries. Ex vivo testing of delivery parameters of the perfusion catheter determined the varying impacts of drug concentration, excipient, delivery pressure and delivery time. Pharmacokinetic analysis of treated rabbit arteries showed the presence of paclitaxel in the arterial segments with an increase in arterial paclitaxel levels with the addition of an excipient. The paclitaxel levels were within the inhibitory concentration range of smooth muscle cell proliferation³². Overall, these results demonstrate the feasibility to deliver and retain paclitaxel within arterial segments.

Non-stent delivery platforms have gathered momentum in recent years due to shortcomings of stents to treat PAD^{22,33}. The most prevalent has been the advent of drug coated balloons which were recently approved by the FDA. The approach of drug coated balloons is to deliver the anti-proliferative drug paclitaxel in a short, single-dose approach to reduce

neointima formation. Clinical trials have shown that effectiveness of these devices, although most of these studies have been predominantly on randomized trials that have compared drug coated balloons to balloon angioplasty in simple and short lesion subsets. Real world, more complex outcomes of drug coated balloons remain relatively unknown. Particulate embolization of the drug coated balloon carrier excipients has been shown cause fibrinoid necrosis in downstream tissue²⁷. Additionally, drug coated balloons have had suboptimal outcomes in BTK studies³⁴. In these scenarios, developing alternative strategies to treat patients with PAD is of significant value.

One of the main commonality of the varying strategies to treat PAD is the use of paclitaxel, a highly potent anti-proliferative that has shown to be effective in the short, single dose approach³⁵. The perfusion catheter shares similar traits with the drug coated balloon as it delivers paclitaxel in a short and single dose manner using established clinical catheter techniques, however, the paclitaxel agent is in liquid form as compared to dry or powdered paclitaxel form. The benefit of such a liquid solution is the ability to deliver anti-proliferative drugs directly into the medial layer, as observed in Figure 3. Additionally, this approach eliminates any potential loss of drug in transit/tracking, allows multiple treatments per patient, and reduces the risk of embolization as observed in drug coated balloon coating/excipient.

In this study, the well-established swine and rabbit arteries were utilized to test the pharmacokinetic profiles of the perfusion catheter^{36,37}. In the ex vivo portion of the study, we varied the loading parameters of the perfusion catheter to determine parameters to maximize paclitaxel retention acutely (1 hour). The paclitaxel concentration selected were 6.0 mg/ml, the maximum (stock) solution of the liquid paclitaxel, and 1.2 mg/ml, the manufacturer recommended dilution of paclitaxel. The 3.6 mg/ml was the median value. We additionally tested the impact of increasing duration of delivery from 2 minutes to 4 minutes. Longer duration times were not further investigated due to clinical setting consideration, as catheter procedures (stents and/or balloons) are typically deployed at 30 seconds to 2 minutes. And lastly, we tested the increase of pressure delivery from 2 atm to 4 atm. Additional delivery pressure increased the risk of drug leakage around the proximal and distal occlusion balloons and was thus not investigated. The overall lack of significance between the varying groups is somewhat expected, as pharmacokinetic data is known to have high variability at acute time points³¹. Despite this outcome, the results of the ex vivo model demonstrated the capability of the perfusion catheter to delivery paclitaxel to arterial lesions using a bench-top model, minimizing the usage of additional resources and animals.

Following the ex vivo studies, a clinically relevant preclinical model was used to further demonstrate the capabilities of the perfusion catheter^{37,38}. The in vivo comparator groups were Group I and Group IV, which represented a drug mixture with and without an excipient with similar ex vivo 1-hour paclitaxel loading conditions. The excipient, the contrast media iohexol, was intended to increase residency of paclitaxel as previous non-stent platform studies had indicated paclitaxel alone is not sufficient for long-term residency^{39,40}. Our results demonstrated a significant decrease in total drug retained in the artery at 3 days as compared to 1 hour in the non-excipient group, whereas, in the drug combined with excipient, drug retention was non-significantly similar. Additionally, the rabbit studies

demonstrated the validity of the ex vivo model as paclitaxel concentration levels at 1 hour of both the excipient (in vivo: 9.00 ± 5.72 ng/mg vs. ex vivo: 15.4 ± 8.6 ng/mg, $p = 0.81$) and non-excipient (in vivo: 10.53 ± 7.33 ng/mg vs. ex vivo: 8.9 ± 6.9 ng/mg, $p = 0.99$) groups were similar.

As mentioned previously, the contrast media iohexol was utilized to enhance paclitaxel delivery. Although other studies have demonstrated similar favorable paclitaxel uptake and retention in the use of contrast media ^{39,40}, the potential mechanism has not been described. Iohexol, a non-ionic contrast agent, possess lipophilic zones, in particular triiodobenzene ring (TIB), that is responsible for the interaction of iohexol to biological sites. The interaction of iohexol to biological sites is essentially hydrophobic in nature, similar to paclitaxel, and has been shown to interact with non-specific binding sites such as serine proteinases ⁴¹. Together, the hydrophilic iohexol which bonds non-covalently to the hydrophobic paclitaxel, increases the potential non-specific binding within the artery and increasing the residency of the paclitaxel. These potential mechanisms may explain the outcome of our results in which an increase in paclitaxel retention was observed following the addition of iohexol. In addition to increasing residency of paclitaxel, the iohexol excipient has one additional benefit of that it can be visualized during the filling of the treatment chamber, which can be used as visual confirmation in a clinical setting by interventionist.

Pressure is the main driving force that delivers the liquid paclitaxel mixture to the medial layer of the target lesion. For this study, pressure was generated by the delivery of the liquid paclitaxel into the treatment chamber through the inflow port (Fig. 2). Pressure generated in the treatment chamber is dependent on the treatment chamber size, arterial compliance, lesion complexity, presence of side branches within the treatment chamber and viscosity of the drug mixture. For the in vivo studies, the measured treatment pressure chamber ranged from was 0.47 to 2.0 atm. This pressure was measured using a built-in fiber optic pressure within the treatment chamber. In clinical settings however, the presence of bifurcations and branches may reduce the treatment chamber pressure during delivery of the drug. To determine if varying treatment pressure chamber impact drug penetration, additional ex vivo studies were performed that delivered the fluorescent paclitaxel at treatment chamber pressures ranging from 0.2 to 1.0 atm. In all cases, drug was able to penetrate into the medial layer, although the depth of penetration was dependent on the treatment chamber pressure (Fig. 5). Further studies on the relationship between treatment chamber pressure, drug viscosity and lesion characteristics are warranted to better understand and optimize local drug delivery using perfusion catheters.

The use of liquid paclitaxel to treat PAD has been utilized in prior studies. Of importance was the THUNDER (local taxane with short exposure for reduction of restenosis in distal arteries) trial, which demonstrated a reduction in 6-month late lumen loss of paclitaxel-coated drug coated balloon as compared to balloon angioplasty ⁴². In the same trial, however, paclitaxel-contrast liquid solution (approximately 100 ml of contrast/paclitaxel medium added to circulation via a catheter injection) had similar results to balloon angioplasty. On the contrary, a more recent study has demonstrated effectiveness of liquid paclitaxel to treat patients with critical limb ischemia ⁴³. In this single-center, single-blind,

randomized trial, patients treated with local liquid paclitaxel (mixed with contrast agent) had less restenosis and amputation as patients treated with drug coated balloon. The liquid paclitaxel, delivered following ultrasound treatment, was delivered stagnantly (using balloon to close of distal outlets) for 60 seconds at the lesion site. These studies highlight differences in delivery mechanism of liquid paclitaxel and its relationship to clinical outcomes.

This study was limited to a healthy animal model and thus did not take into consideration diseased arteries as observed in patients with PAD. The perfusion catheter treated arteries lacking major side branches and bifurcations, which may not represent all clinical settings. Human lesions are more complex and often include fibrosis, calcification, hemorrhage and in most cases require de-bulking using balloons and atherectomy devices. The impact of intact endothelium (non-injured) on paclitaxel-excipient penetration was not determined. Nevertheless, preclinical studies involving healthy arteries are the standard model to determine pharmacokinetic of cardiac and stent-based intervention devices.

Conclusions

This study provides first evidence of the use of an occlusion perfusion catheter to deliver liquid paclitaxel uniformly into the medial wall of an artery using both ex vivo and in vivo models. The results showed that the addition of an excipient increases arterial paclitaxel residency. Further studies are warranted to demonstrate the safety and efficacy profile of the perfusion catheter up to 28 days. Overall, this approach has the potential to improve interventional outcomes and quality of life of millions of patients suffering with PAD.

Acknowledgments

Funding: This work was supported by the American Heart Association [#15SDG25880000]; National Institute of Health [#1R15HL127596], a research grant from the University of South Alabama Office and Research Development; and a research grant from Advanced Catheter Therapies.

References

1. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2007; 33(Suppl 1):S1–75.
2. Tsetis D, Belli AM. Guidelines for stenting in infrainguinal arterial disease. Cardiovascular and interventional radiology. 2004; 27(3):198–203. [PubMed: 15129334]
3. Singh GD, Armstrong EJ, Laird JR. Femoropopliteal in-stent restenosis: current treatment strategies. The Journal of cardiovascular surgery. 2014; 55(3):325–333. [PubMed: 24755700]
4. Scheinert D, Scheinert S, Sax J, et al. Prevalence and clinical impact of stent fractures after femoropopliteal stenting. J Am Coll Cardiol. 2005; 45(2):312–315. [PubMed: 15653033]
5. Rits J, van Herwaarden JA, Jahrome AK, Krievins D, Moll FL. The incidence of arterial stent fractures with exclusion of coronary, aortic, and non-arterial settings. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2008; 36(3):339–345.
6. Babaev A, Hari P, Zavrunova S, Kurayev A. Role of Nitinol Stent Fractures in the Development of In-Stent Restenosis in the Superficial Femoral Artery. Vascular Disease Management. 2016; 1(13):E7–E16.
7. Lin Y, Tang X, Fu W, Kovach R, George JC, Guo D. Stent fractures after superficial femoral artery stenting: risk factors and impact on patency. J Endovasc Ther. 2015; 22(3):319–326. doi:

310.1177/1526602815580783. Epub 1526602815582015 Apr 1526602815580710. [PubMed: 25862365]

8. Caradu C, Brizzi V, Auque H, Midy D, Ducasse E. Sense and nonsense of bare metal stents below the knee. *J Cardiovasc Surg (Torino)*. 2016; 57(5):653–666. Epub 2016 May 2013.
9. Ricco JB, Thanh Phong L, Schneider F, et al. The diabetic foot: a review. *The Journal of cardiovascular surgery*. 2013; 54(6):755–762. [PubMed: 24126512]
10. Sumpio BE, Lee T, Blume PA. Vascular evaluation and arterial reconstruction of the diabetic foot. *Clinics in podiatric medicine and surgery*. 2003; 20(4):689–708. [PubMed: 14636033]
11. Fernandez N, McEnaney R, Marone LK, et al. Predictors of failure and success of tibial interventions for critical limb ischemia. *J Vasc Surg*. 2010; 52(4):834–842. [PubMed: 20619586]
12. Schillinger M, Sabeti S, Loewe C, et al. Balloon angioplasty versus implantation of nitinol stents in the superficial femoral artery. *N Engl J Med*. 2006; 354(18):1879–1888. [PubMed: 16672699]
13. Schillinger M, Sabeti S, Dick P, et al. Sustained benefit at 2 years of primary femoropopliteal stenting compared with balloon angioplasty with optional stenting. *Circulation*. 2007; 115(21): 2745–2749. [PubMed: 17502568]
14. Dick P, Wallner H, Sabeti S, et al. Balloon angioplasty versus stenting with nitinol stents in intermediate length superficial femoral artery lesions. *Catheter Cardiovasc Interv*. 2009; 74(7): 1090–1095. [PubMed: 19859954]
15. Krankenberg H, Schluter M, Steinkamp HJ, et al. Nitinol stent implantation versus percutaneous transluminal angioplasty in superficial femoral artery lesions up to 10 cm in length: the femoral artery stenting trial (FAST). *Circulation*. 2007; 116(3):285–292. [PubMed: 17592075]
16. Laird JR, Katzen BT, Scheinert D, et al. Nitinol stent implantation versus balloon angioplasty for lesions in the superficial femoral artery and proximal popliteal artery: twelve-month results from the RESILIENT randomized trial. *Circulation Cardiovascular interventions*. 2010; 3(3):267–276. [PubMed: 20484101]
17. Kropman RH, Bemelman M, Vos JA, et al. Long-term results of percutaneous transluminal angioplasty for symptomatic iliac in-stent stenosis. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2006; 32(6):634–638.
18. Kudo T, Chandra FA, Ahn SS. Long-term outcomes and predictors of iliac angioplasty with selective stenting. *J Vasc Surg*. 2005; 42(3):466–475. [PubMed: 16171589]
19. Koizumi A, Kumakura H, Kanai H, et al. Ten-year patency and factors causing restenosis after endovascular treatment of iliac artery lesions. *Circulation journal : official journal of the Japanese Circulation Society*. 2009; 73(5):860–866. [PubMed: 19282607]
20. Brodmann M. Prime time for drug eluting balloons in SFA interventions? *The Journal of cardiovascular surgery*. 2014; 55(4):461–464. [PubMed: 24941235]
21. Buszman PP, Tellez A, Afari ME, et al. Tissue uptake, distribution, and healing response after delivery of paclitaxel via second-generation iopromide-based balloon coating: a comparison with the first-generation technology in the iliofemoral porcine model. *JACC Cardiovascular interventions*. 2013; 6(8):883–890. [PubMed: 23968706]
22. De Vries JP, Karimi A, Fiore B, Van Leersum M, Werson DA, Van Den Heuvel DA. First- and second-generation drug-eluting balloons for femoro-popliteal arterial obstructions: update of technique and results. *The Journal of cardiovascular surgery*. 2013; 54(3):327–332. [PubMed: 23640355]
23. Joner M, Finn AV, Farb A, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol*. 2006; 48(1):193–202. [PubMed: 16814667]
24. Smouse HB, nikanorov A, LaFlash D. Biomechanical forces in the femoropopliteal arterial segment. *Endovascular Today*. 2005 Jun.:60–66.
25. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *European heart journal*. 2013; 34(31):2436–2443. [PubMed: 23641007]
26. Beckman JA, Paneni F, Cosentino F, Creager MA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part II. *European heart journal*. 2013; 34(31):2444–2452. [PubMed: 23625211]

27. Kolodgie FD, Pacheco E, Yahagi K, Mori H, Ladich E, Virmani R. Comparison of Particulate Embolization after Femoral Artery Treatment with IN.PACT Admiral versus Lutonix 035 Paclitaxel-Coated Balloons in Healthy Swine. *J Vasc Interv Radiol*. 2016; 27(11):1676–1685. [PubMed: 27641674]
28. Ellis SG, Stone GW, Cox DA, et al. Long-term safety and efficacy with paclitaxel-eluting stents: 5-year final results of the TAXUS IV clinical trial (TAXUS IV-SR: Treatment of De Novo Coronary Disease Using a Single Paclitaxel-Eluting Stent). *JACC Cardiovascular interventions*. 2009; 2(12): 1248–1259. [PubMed: 20129552]
29. Yazdani SK, Sheehy A, Nakano M, et al. Preclinical evaluation of second-generation everolimus- and zotarolimus-eluting coronary stents. *J Invasive Cardiol*. 2013; 25(8):383–390. [PubMed: 23913602]
30. Zhang YL, Bendrick-Peart J, Strom T, Haschke M, Christians U. Development and validation of a high-throughput assay for quantification of the proliferation inhibitor ABT-578 using LC/LC-MS/MS in blood and tissue samples. *Therapeutic drug monitoring*. 2005; 27(6):770–778. [PubMed: 16306853]
31. Gongora CA, Shibuya M, Wessler JD, et al. Impact of Paclitaxel Dose on Tissue Pharmacokinetics and Vascular Healing: A Comparative Drug-Coated Balloon Study in the Familial Hypercholesterolemic Swine Model of Superficial Femoral In-Stent Restenosis. *JACC Cardiovascular interventions*. 2015; 8(8):1115–1123. [PubMed: 26117470]
32. Axel DI, Kunert W, Goggelmann C, et al. Paclitaxel inhibits arterial smooth muscle cell proliferation and migration in vitro and in vivo using local drug delivery. *Circulation*. 1997; 96(2): 636–645. [PubMed: 9244237]
33. Ng VG, Mena C, Pietras C, Lansky AJ. Local delivery of paclitaxel in the treatment of peripheral arterial disease. *European journal of clinical investigation*. 2015; 45(3):333–345. [PubMed: 25615282]
34. Zeller T, Baumgartner I, Scheinert D, et al. Drug-eluting balloon versus standard balloon angioplasty for infrapopliteal arterial revascularization in critical limb ischemia: 12-month results from the IN.PACT DEEP randomized trial. *J Am Coll Cardiol*. 2014; 64(15):1568–1576. doi: 1510.1016/j.jacc.2014.1506.1198. [PubMed: 25301459]
35. Speck U, Cremers B, Kelsch B, et al. Do pharmacokinetics explain persistent restenosis inhibition by a single dose of paclitaxel? *Circulation Cardiovascular interventions*. 2012; 5(3):392–400. [PubMed: 22619258]
36. Schwartz RS, Chronos NA, Virmani R. Preclinical restenosis models and drug-eluting stents: still important, still much to learn. *J Am Coll Cardiol*. 2004; 44(7):1373–1385. [PubMed: 15464316]
37. Schwartz RS, Edelman E, Virmani R, et al. Drug-eluting stents in preclinical studies: updated consensus recommendations for preclinical evaluation. *Circulation Cardiovascular interventions*. 2008; 1(2):143–153. [PubMed: 20031669]
38. Virmani R, Kolodgie FD, Farb A, Lafont A. Drug eluting stents: are human and animal studies comparable? *Heart (British Cardiac Society)*. 2003; 89(2):133–138. [PubMed: 12527658]
39. Scheller B, Speck U, Romeike B, et al. Contrast media as carriers for local drug delivery. Successful inhibition of neointimal proliferation in the porcine coronary stent model. *European heart journal*. 2003; 24(15):1462–1467. [PubMed: 12909076]
40. Scheller B, Speck U, Schmitt A, Bohm M, Nickenig G. Addition of paclitaxel to contrast media prevents restenosis after coronary stent implantation. *J Am Coll Cardiol*. 2003; 42(8):1415–1420. [PubMed: 14563585]
41. Prange T, Gaudey F, Ohanessian J, et al. Angiography contrast agents interact with serine proteinases. The molecular structure of the model system elastase/iohexol. *FEBS Lett*. 1995; 357(3):247–250. [PubMed: 7835421]
42. Tepe G, Zeller T, Albrecht T, et al. Local delivery of paclitaxel to inhibit restenosis during angioplasty of the leg. *N Engl J Med*. 2008; 358(7):689–699. doi:10.1056/NEJMoa0706356. [PubMed: 18272892]
43. Gandini R, Del Giudice C. Local Ultrasound to Enhance Paclitaxel Delivery After Femoral-Popliteal Treatment in Critical Limb Ischemia: The PACUS Trial. *JACC Cardiovasc Interv*. 2016; 9(20):2147–2153. doi:10.1016/j.jcin.2016.2107.2019. [PubMed: 27765310]

Figure 1.

Schematic diagram of the ex vivo bioreactor system. (A-E) A computer controlled gear pump (B) generated physiological flow through a port (C) of CO₂ incubator. The culture medium then passed through a low-pass filter and through the lumen of the explanted arteries in the bioreactor housing compartment (D) and into the flow reservoir (E). The arrows indicate direction of flow. (G-H) Recorded graphs of pressure (G) and flow (H) within the ex vivo vascular system.

Figure 2.

Schematic illustration of the perfusion catheter. Two occluding balloons are deployed to momentarily stop circulating flow to a portion of a vessel. Trapped blood within the perfusion chamber is then flushed with saline. Drug can be delivered through the inflow port. The outflow port can be closed and drug delivered to the lesion with external pressure. The delivery pressure is measured by a fiber optic pressure sensor located at the inlet port. Following the delivery of the drug, the remaining drug in the chamber is cleared through the outflow port to ensure no additional drug is introduced into the circulatory system.

Figure 3.

Deployed perfusion catheter. (Top) Image of the perfusion catheter system deployed within the explanted pig artery within the ex vivo bioreactor system. Due to the optical clarity of the system, the proximal and distal occlusion balloons are clearly visible and can be identified in the artery. (Bottom) Confocal images of 1 hour treated explanted pig arteries showing differences in penetration between Flutax-1 with and without excipient. Red arrow denotes green fluorescence associated with the Flutax-1 agent (L=lumen). (yellow bar – 500 μm)

Figure 4.

Total arterial paclitaxel levels of the perfusion catheter treated arteries segments with and without the contrast agent excipient. (right) Angiogram of the perfusion catheter during delivery. The red arrow indicates the treatment chamber filled with paclitaxel and the contrast excipient.

Figure 5. The impact of treatment chamber pressure on drug penetration ex vivo. Confocal images of explanted pig arteries showing differences in Flutax-1 penetration for treatment chamber pressures of 1 atm (A), 0.6 atm (B) and 0.2 atm (C). Red arrow denotes green fluorescence associated with the Flutax-1 agent (L=lumen). (yellow bar – 250 μm)

Table 1

Pharmacokinetics of treated arteries within the bench-top model.

	# of treated vessels	Time Point (hr)	Duration (min)	Delivery Pressure (atm)	Paclitaxel Concentration (mg/ml)	Addition of Excipient	Arterial Paclitaxel (ng/mg) ¹	Total Paclitaxel (µg) ²
Group I	4	1	2	2	1.2	No	8.9±6.9	7.55±9.29
Group II	4	1	2	2	1.2	Yes	13.2±9.5	11.38±9.73
Group III	4	1	2	2	3.6	No	5.1±3.9	2.58±1.55
Group IV	4	1	2	2	3.6	Yes	15.4±8.6	11.30±14.39
Group V	4	1	2	2	6.0	No	21.2±10.4	16.28±11.95
Group IIIa	4	1	2+2 (4)	2	3.6	Yes	20.7±4.70	10.91±4.10
Group IIIb	4	1	2	4	3.6	Yes	25.9±11.28	12.29±5.70

¹ Arterial paclitaxel is the amount of paclitaxel measured in harvested (treated) tissue normalized to the tissue weight (concentration)

² Total paclitaxel is the amount of paclitaxel measured in the harvested (treated) tissue

Table 2

Pharmacokinetics of treated rabbit iliac arteries.

# of samples	Time Point (hr)	Duration (min)	Delivery Pressure (ATM)	Pacitaxel Concentration (mg/ml)	Addition of Excipient	Arterial Pacitaxel (ng/mg)	Paired comparisons, p < 0.05	Total Pacitaxel (ng)	Paired comparisons, p < 0.05
(n=3)	1	2	2	3.6	Yes	9.00±5.72		107.67±62.06	
(n=3)	24	2	2	3.6	Yes	6.56±1.85	ns	198±95.6	ns
(n=3)	72	2	2	3.6	Yes	2.04±2.44		40.07±23.04	
(n=3)	1	2	2	1.2	No	10.53±7.33		246.9±120.3	
(n=3)	24	2	2	1.2	No	2.09±1.44	ns	12.13±10.12	1 hr vs. 1 day 1 hr vs. 3 days
(n=3)	72	2	2	1.2	No	0.45±0.46		2.92±2.91	

ns - non significant