Pharmaceutical Research, Vol. 23, No. 4, April 2006 (© 2006)
DOI: 10.1007/s11095-006-9746-2

Research Paper

Perivascular Tissue Pharmacokinetics of Dipyridamole

Kosalaram Goteti,[1] Takahisa Masaki,[2] Tadashi Kuji,[2] John K. Leypoldt,[2,3,4] Alfred K. Cheung,[2,5] and Steven E. Kern[1,2,3,6]

Received September 2, 2005; accepted December 13, 2005

Purpose. The tissue diffusivity (D_g) and partitioning (K) for dipyridamole were determined and a model was developed to examine the relationship between perivascular dose and local dipyridamole tissue concentrations.

Methods. Experiments were performed using an *in vitro* perfusion apparatus that recirculated buffer through different graft samples or normal porcine femoral arteries and veins. The grafts or blood vessels were immersed in a compartment containing Krebs–Henseleit (KH) buffer and dipyridamole (30 μg/mL). The recirculating buffer was sampled at multiple time points and dipyridamole was assayed. Estimates of the effective diffusivity (D_g) and partition coefficient (K) of the drug in the vessel wall were determined and used to simulate dipyridamole tissue concentration after perivascular delivery.

Results. Dipyridamole diffusivity within native femoral veins ($D_g = 3.87 \pm 0.93 \times 10^{-6}$ cm^2/s) was approximately twice that within femoral arteries ($D_g = 2.06 \pm 0.79 \times 10^{-6}$ cm^2/s, $p < 0.01$). Explanted grafts showed the lowest diffusivity. Partition coefficients of femoral arteries ($K = 4.11 \pm 0.99$) were higher than those of femoral veins ($K = 2.05 \pm 0.85$, $p < 0.01$) and explanted graft ($K = 0.89 \pm 0.56$, $p < 0.01$).

Discussion. The results demonstrate that local drug kinetics vary greatly for different types of blood vessels and grafts. The pharmacokinetic parameters and resulting computational simulations are helpful in exploring perivascular drug delivery strategies.

KEY WORDS: local pharmacokinetics; partition coefficient; perivascular tissue pharmacokinetics near hemodialysis vascular access; synthetic PTFE grafts; vascular tissue permeability.

INTRODUCTION

The main cause of stenosis of the hemodialysis vascular access is neointimal hyperplasia, which is the result of the migration and proliferation of vascular smooth muscle cells (VSMCs) from the media to the lumen of blood vessels (1–4). Systemic administration of antiproliferative drugs has been largely unsuccessful in preventing neointimal hyperplasia in animal studies. For example, the systemic administration of dipyridamole failed to prevent neointimal hyperplasia in polytetrafluoroethylene (PTFE) carotid interposition grafts in goats (5) and iliac arteriovenous interposition grafts in dogs (6). In contrast, high concentrations of heparin, when delivered locally, reduced neointimal hyperplasia in a rabbit model of PTFE graft (7), suggesting that the local delivery of antiproliferative drugs may be a better approach to inhibit neointimal hyperplasia in hemodialysis PTFE grafts. Indeed, we have recently demonstrated that local perivascular delivery of paclitaxel to arteriovenous PTFE grafts using a novel drug delivery system inhibited neointimal hyperplasia at both arterial and venous anastomoses in a dog model (8).

Local perivascular drug delivery has been proposed to inhibit neointimal hyperplasia in arterial tissues (9,10). Lovich and Edelman have shown that the majority of heparin deposited perivascularly entered the arterial wall by direct diffusion (11). An overview of various drug transport pathways shows that the efficacy of perivascular delivery around hemodialysis vascular access would be determined by drug diffusion into the PTFE graft, the vein, and the artery (Fig. 1). The precise mechanisms by which drugs are transported from the depot via these pathways, however, seem to be dependent at least in part upon the characteristics of the drug and have not been extensively studied. Drug transport in tissues is often characterized using two parameters: diffusivity and partition coefficient (12,13). Effective diffusivity or permeance represents the rate of drug penetration across these pathways, whereas the partition coefficient evaluates the degree of drug binding to the tissue of interest.

[1] Department of Pharmaceutics & Pharmaceutical Chemistry, University of Utah, 421 Wakara Way, #318, Salt Lake City, Utah 84108, USA.
[2] Department of Medicine, University of Utah, Salt Lake City, Utah, USA.
[3] Department of Bioengineering, University of Utah, Salt Lake City, Utah, USA.
[4] Research Services, Veterans Affairs Salt Lake City Healthcare System, Salt Lake City, Utah, USA.
[5] Medical Services, Veterans Affairs Salt Lake City Healthcare System, Salt Lake City, Utah, USA.
[6] To whom correspondence should be addressed. (e-mail: Steven.Kern@hsc.utah.edu)

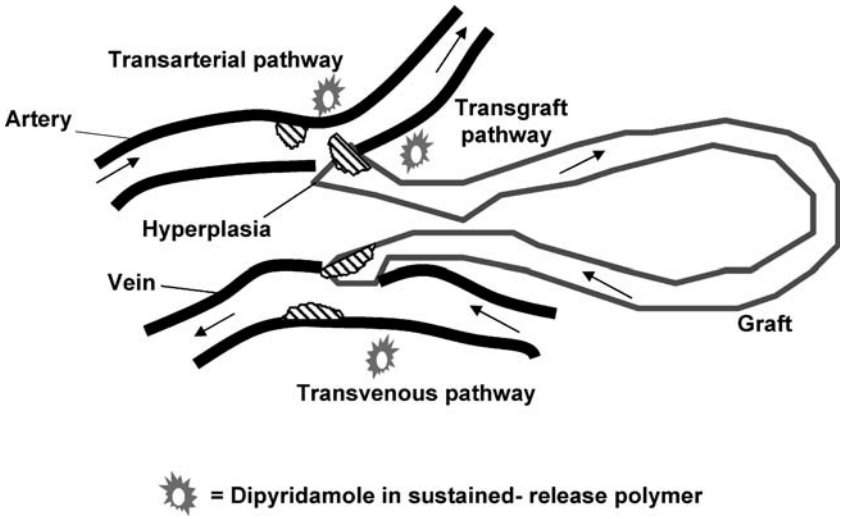

Fig. 1. Proposed perivascular pathways of dipyridamole transport around hemodialysis vascular access.

In this study we evaluated the effect of perivascularly administered drug on the local concentrations of dipyridamole surrounding the hemodialysis vascular graft. We first determined both diffusivities and partition coefficients for the PTFE graft and the native artery and vein *in vitro*. Then, we developed a simple tissue pharmacokinetic model based on principles of mass transfer to illustrate the importance of diffusivities and partition coefficients on local drug concentrations. Dipyridamole is selected as the test drug because it possesses antiproliferative effects (14–16). In addition, continuous perivascular delivery of this drug has been shown to be effective in inhibiting neointima formation in a rabbit model of postangioplasty carotid artery stenosis (17) as antiplatelet properties. These pharmacokinetic data and principles will facilitate the optimization of local drug delivery strategies for preventing vascular access stenosis.

MATERIALS AND METHODS

Chemicals

Dipyridamole [MW = 504.6, log P = 2.74, solubility = 2.77 (870 mg/L)], Roswell Park Memorial Institute (RPMI) culture medium, KH buffer, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Dipyridamole Assay

Concentrations of dipyridamole in ammonium chloride buffer (pH 10) were measured using a fluorescence spectrophotometer (model no. F-4010, Hitachi, Tokyo, Japan) with emission and excitation wavelength of 495.0 and 412.2 nm, respectively (18).

In Vitro Perfusion Studies to Determine Drug Diffusivity with Vessel/Graft Walls

A perfusion apparatus was assembled to hold either native vessels or expanded polytetrafluoroethylene (ePTFE) hemodialysis graphs for studying the transit of dipyridamole across the vessel or graft wall (Fig. 2). Each end of the vessel or graft was slid over the top of a tubing connection and placed inside a Plexiglas chamber of the perfusion apparatus. The chamber, or perivascular compartment, was filled with a sterilized solution of dipyridamole (30 μg/mL) dissolved in KH buffer and sealed tight with screws to avoid any leakage. Two sampling ports along its length allowed access for drug concentration measurements.

The tubing connections, which held the graft or vessel, were coupled to a peristaltic pump (model no.7401, Drake-Willock, Portland, OR, USA) and fluid reservoir that circulated sterilized KH buffer at a flow rate of 100 mL/min through the graft or vessel. This comprised the endovascular compartment. The volume of the perivascular compartment was 100 mL and that for the endovascular compartment including the reservoir was 250 mL. Oxygen (95%) and carbon dioxide (5%) were bubbled continuously into the

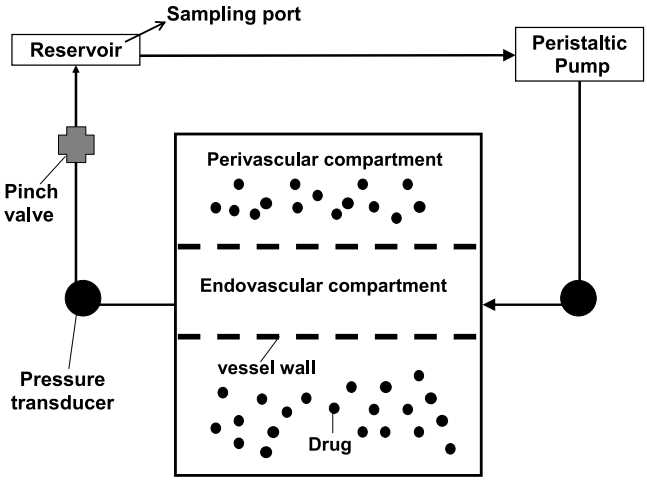

Fig. 2. *In vitro* perfusion apparatus. The apparatus was custom made for the study of transvascular kinetics of dipyridamole across grafts or native vessels.

apparatus to maintain the viability of cells within the vessels. The KH buffer in the endovascular compartment was maintained at 37°C and recirculated using a peristaltic pump while drug transport from the perivascular compartment to the vessel lumen was allowed to occur.

For most experiments, the mean endovascular pressure was controlled by a resistor downstream of the vessel or graft and was maintained at 90 mmHg for grafts or arteries and 20 mmHg pressure for veins. To assess the contribution of convective forces to drug transit across the vessels, a series of experiments were conducted with the endovascular to perivascular pressure gradient fixed at either $\Delta P = 0$ or 80 mmHg for arteries and grafts, respectively, and $\Delta P = 10$ mmHg for veins.

Samples (1 mL) of the KH buffer in the endovascular compartment were obtained from the reservoir and from the perivascular compartment through the ports in the chamber at selected time points up to 24 h. To normalize the transport parameters (see below), the thickness of each vessel used in these *in vitro* perfusion experiments was measured using micrometer scale under a light microscope.

Graft and Vessel Samples

Perfusion studies were conducted with ePTFE hemodialysis grafts (6 mm internal diameter and 5 cm in length, Impra-Bard®, Tempe, AZ, USA), explanted ePTFE grafts taken from a porcine animal model, and normal arterial and venous vessels also from a porcine animal model. The ePTFE grafts were pretreated with blood to mimic *in vivo* situations and to remove air in the pores within graft walls, which provides a great resistance to drug transport (19). An intraluminal pressure of approximately 200 mmHg was briefly applied to degas the grafts by recirculating heparinized blood through the lumen of the PTFE graft. After the air had been expelled, heparinized whole blood was recirculated through the lumen at 400 mL/min for 60 min with a mean intraluminal pressure of 90 mmHg. This graft, referred to as the blood-treated graft, was then immersed in heparinized whole blood for an additional 5 h before testing in the perfusion apparatus.

Explanted ePTFE grafts were removed from the porcine model after 4 weeks' placement between the carotid artery and jugular vein of pigs. All animal procedures and care were performed in accordance with the *Principles of Laboratory Animal Care* and the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996) and approved by the respective Institutional Animal Care and Use Committees (IACUC) of the Veterans Affairs Salt Lake City Healthcare System and the University of Utah. Yorkshire cross domestic swine of either gender, weighing 30–50 kg, were used. At 4 weeks postimplantation, the pigs were euthanized using phenobarbital and segments (3.5–5 cm in length) of the ePTFE grafts were explanted under aseptic conditions and stored in RPMI buffer until testing.

Normal femoral arteries or veins were also isolated from each euthanized pig. To maintain the length and cross-sectional area of the explanted native vessels, a sterilized tubing connector was specifically manufactured at the Utah Artificial Heart Laboratory (Fig. 3). The blood vessels were connected to these tubing connectors by ligating with 4-0 silk suture. These connectors were held at 2-cm distance by a screw that prevented the longitudinal collapse of the vessel. After ligation, the vessels were dissected and the whole system was placed in RPMI medium. The vessel was mounted on the *in vitro* perfusion apparatus immediately after explantation. Leaks from the vessel were checked by injecting KH buffer to one end of the vessel by use of a syringe, with the other end closed. The perfusion experiment was then started immediately.

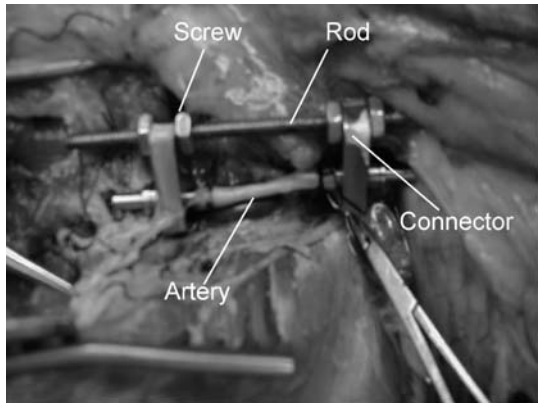

Fig. 3. Connectors used to harvest native porcine femoral vessels. This apparatus was devised to maintain the length of the isolated femoral arteries or femoral veins by the rod following ligation to perform subsequent *in vitro* perfusion studies.

Partition Coefficient Determination

At the completion of experiments in the perfusion apparatus, the vessel or graft was rinsed with KH buffer and surface dried before being weighed and later homogenized in 100% ethanol for 5 min. The homogenate was centrifuged and the supernatant containing dipyridamole was extracted from the vessel and assayed for drug concentration. The partition coefficient was calculated by dividing the dipyridamole concentrations present within the graft/vessel by the dipyridamole concentration in the perivascular compartment at the end of the 24-h perfusion studies.

Tissue Experiments to Determine Partition Coefficients

Because partition coefficient values are typically obtained after equilibration has been achieved, tissue culture experiments were performed to compare the values of partition coefficient obtained from *in vitro* perfusion experiments. Grafts or native blood vessels of known weight were incubated with dipyridamole (30 μg/mL) dissolved in KH buffer at 37°C in 24-well plates. To determine if 24 h of equilibration was sufficient to measure partition coefficient values we performed additional experiments for grafts or native blood vessels for 72 h. After 24 or 72 h of equilibration, the vessel or grafts were removed from the 24-well plates; the concentration of dipyridamole remaining in the wells was measured. The vessel was rinsed with KH buffer, dried, and the amount of dipyridamole deposited onto the vessel was measured after ethanol extraction as described above. The partition coefficient was calculated by dividing the dipyridamole concentration present within the vessel by the bulk dipyridamole concentration remaining in the 24-well plate.

For all the *in vitro* studies, the total drug used in the study was accounted for using a mass balance approach. Measured concentrations in tissue and buffer solution used in the experiment were determined and the total volume was used to account for the amount of drug present. This was compared to the total amount of drug used in the particular *in vitro* trial.

Drug Diffusion Parameters (Drug Diffusivity and Partition Coefficients)

Drug transport parameters were calculated across the various grafts and blood vessels by mass balance and Fick's laws of diffusion (19). The kinetics for the appearance of drug in the endovascular compartment is given by Eq. (1):

$$AP_e\big(C_p(t) - C_e(t)\big) = V_e \frac{dC_e(t)}{dt} \tag{1}$$

where P_e is the permeability through the vessel/graft and A is the surface area of the vessel/graft available for diffusion and was calculated as $2\pi Rh$ (where R is the outer radius and h is the length of the vessel or graft used for perfusion studies). The volume of the endovascular compartment is V_e (250 mL) and $C_p(t)$ and $C_e(t)$ are the concentrations of drug at time t in the perivascular compartment and endovascular compartment, respectively. The initial concentration of dipyridamole in the endovascular compartment was set to zero [$C_e(0) = 0$ μg/mL] and $dC_e(t)/dt$ is the change of concentration in the endovascular compartment with time t. Mass balance of the drug in the *in vitro* perfusion apparatus at any time t is given by Eq. (2):

$$V_pC_p(0) = V_pC_p(t) + V_gC_g(t) + V_eC_e(t) \tag{2}$$

where $C_g(t)$ is the concentration of drug within the vessel at time t and $C_p(0)$ is the drug concentration in the perivascular compartment at time zero [$C_p(0) = 30$ μg/mL]. V_p (100 mL) and V_g are the volume of the perivascular compartment and vessel wall, respectively. The partition coefficient (K) of the drug into the vessel at any time t would be given by Eq. (3). These values were obtained from the tissue culture experiments after equilibration between the vessel/graft and perivascular compartments.

$$K = \frac{C_g(t)}{C_{bulk}(t)} \tag{3}$$

where C_{bulk} was the bulk perivascular concentration surrounding the tissue of interest in the 24-well plates (20). Solving the above Eqs. (1), (2), and (3), we obtain a monoexponential Eq (4):

$$\frac{C_e(t)}{C_p(0)} = \frac{V_p}{V_p + V_e + 2V_gK}\left(1 - e^{-\left[\frac{AP_e}{V_gK+V_p}\right]\left[\frac{V_p+V_e+2V_gK}{V_e}\right]t}\right) \tag{4}$$

Linear approximation of the above monoexponential Eq. (4) by Taylor series expansion gives Eq. (5):

$$\frac{C_e(t)}{C_p(0)} = \frac{AP_e}{(V_gK + V_p)}\frac{V_p}{V_e}t \tag{5}$$

where the volume of the vessel was calculated as $V_g = \pi(R^2 - r^2)h$ (where R is the outer and r is the inner radius and h is the length of the vessel used in the *in vitro* perfusion apparatus).

The partition coefficient K was calculated using the data obtained from the perfusion experiments and compared with that calculated using data from the tissue culture experiments. Equation (5) was fitted to the time-dependent endovascular concentration of dipyridamole to calculate P_e using the linear regression (Sigma Plot 2000, Point Richmond, CA) (Fig. 4). The effective diffusivity or permeance (D_g) of the vessel/graft was obtained by Eq. (6) to account for the differences in thickness between various grafts and vessels:

$$D_g = P_e(R - r). \tag{6}$$

Tissue Pharmacokinetics Model

Mathematical modeling provides a predictive tool for designing perivascular delivery systems. Because our *ex vivo* results showed that the perivascularly applied drug transport is mostly dominated by diffusion via most pathways, convection was neglected for all our simulations. The modeling process of perivascular delivery depends on two important factors: 1) modeling drug release from the drug delivery system and 2) modeling drug transport within the vessel/graft walls (Fig. 5).

Modeling Drug Release from Perivascular Drug Delivery System

The diffusivity of drug from drug delivery system was obtained by *in vitro* studies using equation. *In vitro* release studies of dipyridamole in a novel thermosensitive polymer were performed earlier (21) (Fig. 6). The polymer (ReGel, Macromed, Sandy, UT) is a triblock copolymer of PGA–PLGA–PGA that has been used for the local delivery of paclitaxel. From these *in vitro* release profiles, the effective diffusivity of dipyridamole within this novel thermosensitive polymer (D_p) was determined using Eq. (7) (Scientist, Micromath scientific software, UT) (22). The drug and polymer were assumed to be a homogenous matrix.

$$\text{Relative release} = 100\left[1 - \frac{8}{\pi^2}\sum_{j=1,3,5}^{\infty}\frac{e^{-Dpt(j\pi/L)^2}}{j^2}\right] \tag{7}$$

where *Relative Release* was the percentage cumulative release of drug from the polymer and L (0.2 mm) was the thickness of the polymer drug layer during our *in vitro* release experiments. The transport and release of drug from this homogenous polymer matrix in Cartesian coordinates was given by Eq. (8):

$$\frac{\partial C_{pol}}{\partial t} = D_{p_x}\frac{\partial^2 C_{pol}}{\partial x^2} + D_{p_y}\frac{\partial^2 C_{pol}}{\partial y^2} + D_{p_z}\frac{\partial^2 C_{pol}}{\partial z^2} - k_{pol}C_{pol} \tag{8}$$

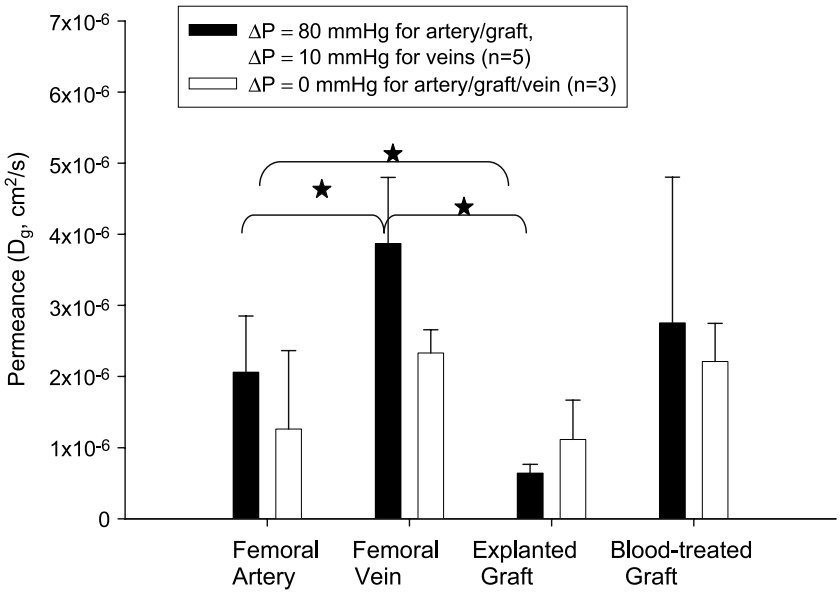

Fig. 4. Permeance (D_g) across the PTFE graft or vessels and the subsequent effect of physiologic pressure gradients between endovascular and perivascular compartment. The endovascular pressure was constant at 90 mmHg for arteries and grafts and 20 mmHg for veins. The positive hydrostatic pressure gradient due to difference between endovascular and perivascular compartment pressure was varied from ΔP = 80 mmHg for arteries and ΔP = 10 mmHg for veins (filled bars) to ΔP = 0 mmHg for all grafts and vessels (open bars). ANOVA shows significant differences ($\bigstar P < 0.01$) in D_g between vessels and grafts. There was no significant difference ($dp > 0.01$) in D_g due to the pressure gradient (ΔP = 80 mmHg and ΔP = 0 mmHg) for arteries and grafts.

where C_{pol} was the concentration of drug within polymer matrix and k_p is the rate of drug loss to the surrounding tissues and extravascular capillaries from the polymer matrix. Initial conditions within a homogenous drug–polymer matrix were assumed to be $C_{pol} = C_{pol}(0) = 10$ mg/mL at time $t = 0$. Diffusivities of drug with drug–polymer matrix were assumed to be isotropic (i.e., $D_p = D_{p_x} = D_{p_y} = D_{p_z}$).

Modeling of Drug Transport within Vessel Walls

The diffusional transport and distribution of the drug within vessel/graft wall in Cartesian coordinates was modeled using Eq. (9):

$$\frac{\partial C_g}{\partial t} = D_{g_x}\frac{\partial^2 C_g}{\partial x^2} + D_{g_y}\frac{\partial^2 C_g}{\partial y^2} + D_{g_z}\frac{\partial^2 C_g}{\partial z^2} - k_g C_g \qquad (9)$$

where C_g was the concentration of drug within the vessel wall and k_p is the rate of drug loss due to metabolism from the vessel wall. Initial conditions for drug concentration in the vessel wall was taken as negligible [$C_g = C_g(0)$ at time $t = 0$] and diffusivities were assumed to be isotropic within vessel walls (i.e., $D_g = D_{g_x} = D_{g_y} = D_{g_z}$). The mean drug diffusivities with positive pressure gradient were used in all simulations.

Due to discontinuities at the interface, the system of equations was solved using a stiff method for attaining continuity of fluxes. The boundary condition at the interface of polymer/tissue interface was given by Eq. (10):

$$J = P_e\left(\frac{C_g}{K} - C_p\right) \qquad (10)$$

where J was the flux at the interfaces. Similarly, the boundary condition at the interface of tissue/polymer interface was given by Eq. (11):

$$J = P_e\left(C_p - \frac{C_g}{K}\right) \qquad (11)$$

The boundary condition at the interface of tissue/blood was given by Eq. (12):

$$J = P_e\left(C_{bld} - \frac{C_g}{K}\right) \qquad (12)$$

where C_{bld} was defined as the concentration of the drug in blood and was assumed negligible ($C_{bld} = 0$) based on our previous *in vivo* studies (8). All other boundaries were assumed to be symmetric with no flux.

All the computational simulations were carried out using FEMLAB® (Cosmol Inc, Burlington, MA, USA). The finite element method was used to solve these equations using a built in 3D chemical engineering diffusion mode in time-dependent module. The model was constructed using the built-in draw mode (Fig. 5). The thickness ($R-r$) of graft, artery, and vein were taken as 0.7, 0.7, and 0.3 mm,

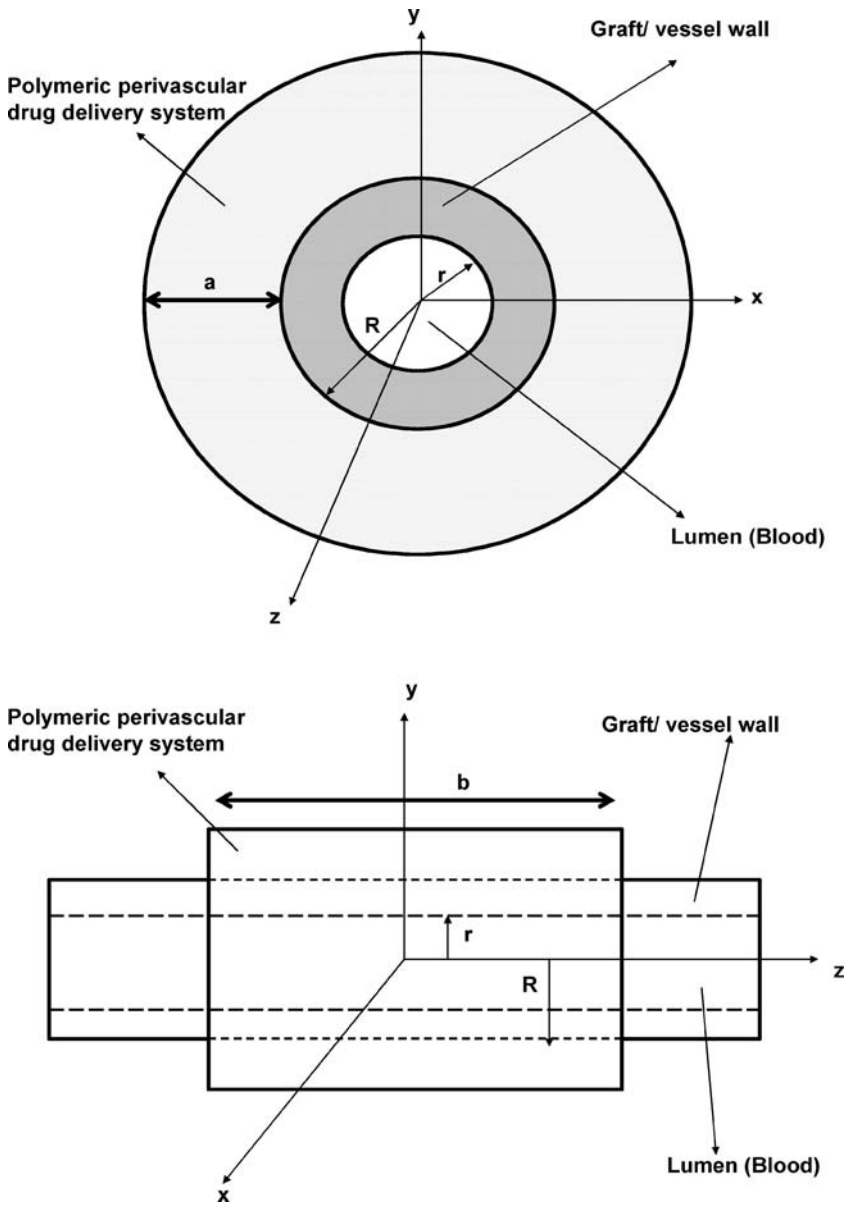

Fig. 5. Schematic of the cross section of drug transport into graft/vessel wall following perivascular drug delivery in Cartesian coordinates. The thickness of graft or vessel wall is given by $(R - r)$, b is the surface area of perivascular application, and a is the thickness of the drug delivery system.

respectively, based on microscopic examination. The length of the vessels/graft was 4 cm. The meshing for all the 3D structures was carried out using the extremely coarse option. The amount of drug within the vessel walls was obtained using the subdomain integration function using the post mode.

Statistical Analyses

All statistical analyses were performed using Splus 5.1 (Mathsoft, Seattle, WA, USA). All data are expressed as mean ± SD. Effective drug diffusivities and partition coefficients obtained under the various experimental conditions were compared using ANOVA followed by the Bonferroni–Dunn correction. Values of $p < 0.01$ are considered statistically significant.

RESULTS

Permeance or Effective Diffusivity within Vessel Wall (D_g)

All the perfusion studies were carried out at an endovascular pressure of 90 mmHg for explanted, blood-treated, and native porcine femoral arteries, and 20 mmHg for native porcine femoral veins to mimic *in vivo* conditions. The time-dependent changes in concentration (kinetic profile) of dipyridamole in the endovascular compartment were obtained for explanted, blood-treated native arteries ($n = 5$)

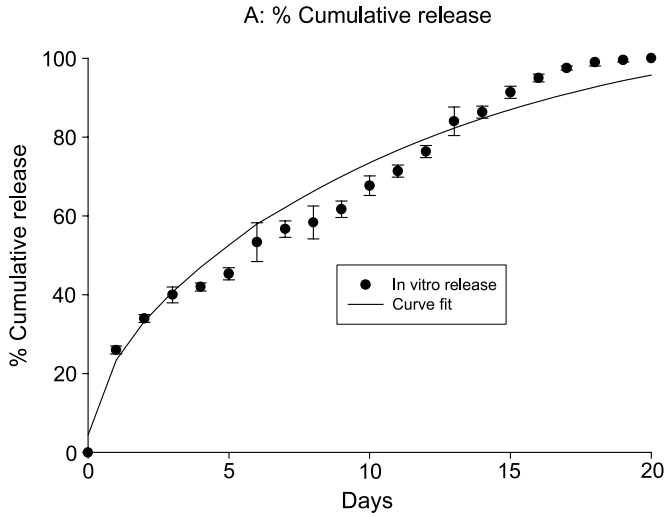

Fig. 6. *In vitro* release profiles of dipyridamole from the drug delivery system and curve fit to obtain the diffusivity within polymer.

at ΔP = 80 mmHg and veins (n = 5) at ΔP = 10 mmHg, where "ΔP is the pressure gradient (endovascular pressure − perivascular pressure) to mimic *in vivo* conditions.

To assess the effect of drug diffusivity without any pressure gradient (ΔP = 0 mmHg) between the endovascular and perivascular compartment, we studied explanted blood-treated grafts (n = 3) and native arteries (n = 3) at perivascular pressure of 90 and 20 mmHg for native veins (n = 3). Permeance or effective diffusivities calculated from the obtained profiles with different pressure gradients ΔP for vessels and grafts are shown in Fig. 4. Changes in ΔP from ΔP = 80 mmHg to ΔP = 0 mmHg did not significantly alter effective diffusivities for arteries, explanted graft, blood-treated graft, and buffer-treated graft. A change of pressure gradient from ΔP = 0 to 10 mmHg, however, caused a 66.1% increase in the effective diffusivities of dipyridamole within veins ($p < 0.01$). There was significant difference in effective diffusivities ($p < 0.01$) among the artery, vein, and explanted grafts for all different pressure gradient ΔP conditions.

For conditions with a positive pressure gradient ΔP that mimic physiological conditions, effective diffusivity of dipyridamole within femoral veins [$D_g = (3.87 \pm 0.93) \times 10^{-6}$ cm^2/s, $p < 0.01$]) was significantly greater than that seen with femoral arteries [$D_g = (2.06 \pm 0.79) \times 10^{-6}$ cm^2/s, $p < 0.01$], whereas the effective diffusivities of arteries were significantly greater than that of the explanted grafts [D_g = (0.64 ±

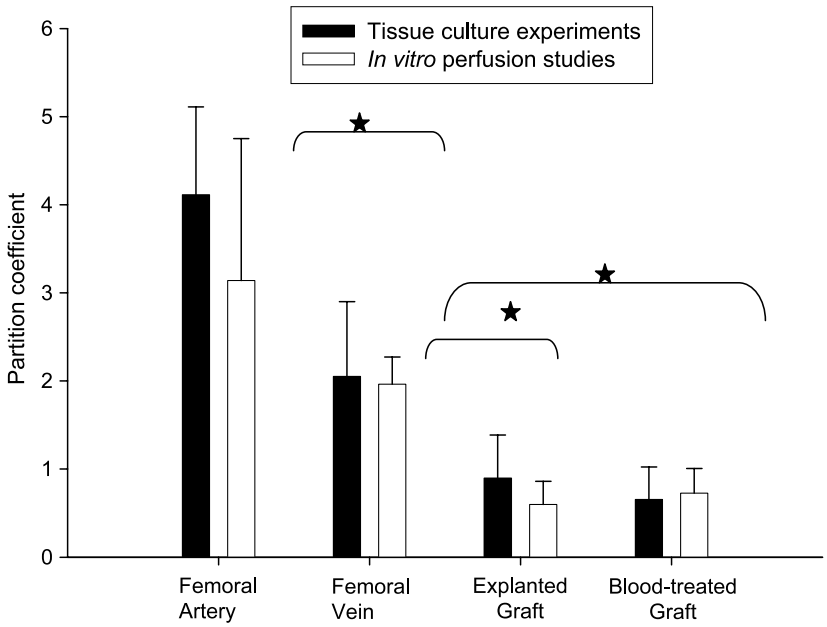

Fig. 7. Partition coefficients (K) of dipyridamole across 1 g of grafts or native vessels obtained from *in vitro* perfusion studies and tissue culture experiments. ANOVA shows significant differences (★$p < 0.01$) in K values between different types of vessels and grafts. There were no significant differences in K values between perfusion experiments and tissue culture experiments for each type of vessel or graft.

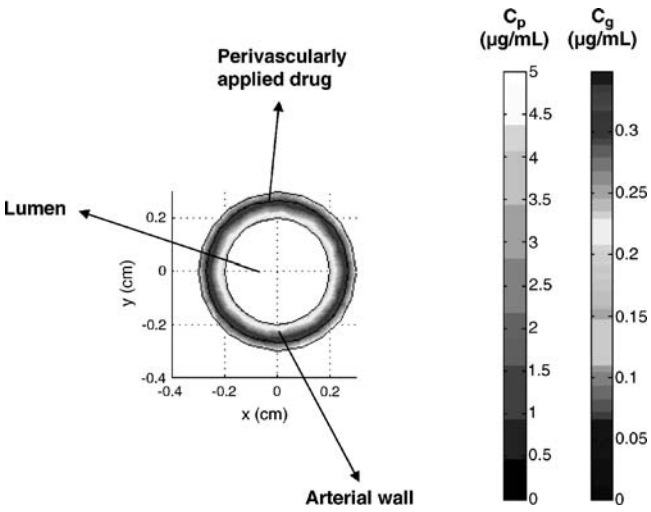

Fig. 8. Simulated contours of dipyridamole concentration within vessel walls (C_g) and polymer matrix (C_p) following perivascular drug administration. The drug is simulated to be placed as a cylinder of uniform thickness on the outside of the vessel. The drug layer is indicated by the external region of the cylinder cross section.

$0.12) \times 10^{-6}$ cm^2/s, $p < 0.01$]. Among the various types of grafts at positive pressure gradient ΔP, the mean drug diffusivities within the blood-treated grafts were greater [$D_g = (2.75 \pm 2.01) \times 10^{-6}$ cm^2/s] than those of the explanted grafts.

Partition Coefficient

The mean partition coefficients (K) of dipyridamole into femoral arteries obtained from the perfusion studies were not significantly different from those obtained from tissue culture studies. Similarly, the partition coefficients of dipyridamole into veins and grafts obtained from the perfusion studies were also not statistically different from the corresponding values obtained from the tissue culture studies (Fig. 7). There were, however, significant differences in partition coefficients ($p < 0.01$) between arteries, veins, and grafts. For example, in tissue culture experiments, the partition coefficient of dipyridamole into arteries (4.11 ± 0.99) was significantly greater than that of the veins (2.05 ± 0.85; $p < 0.01$), whereas the partition coefficient of dipyridamole into veins was significantly greater than those of the explanted (0.89 ± 0.49; $p < 0.01$) and blood-treated grafts (0.65 ± 0.37; $p < 0.01$) (Fig. 7).

Tissue Pharmacokinetic Computational Model

The cumulative release profile of the dipyridamole from the polymer is shown in Fig. 6. The diffusivity of dipyridamole within the polymer ($D_p = 1.8 \times 10^{-8}$ cm^2/s) was obtained by curve fit ($r^2 = 0.9$). Figure 8 shows the typical contours of the drug concentrations within the polymer and graft/vessel walls. Sensitivity analysis, which determines the changes in output parameters due to changes in model parameters, was performed. This distribution within vessel walls (output parameter) depended on various input model parameters like the surface area of perivascular application, the relative rate of drug loss to the surrounding tissues, and

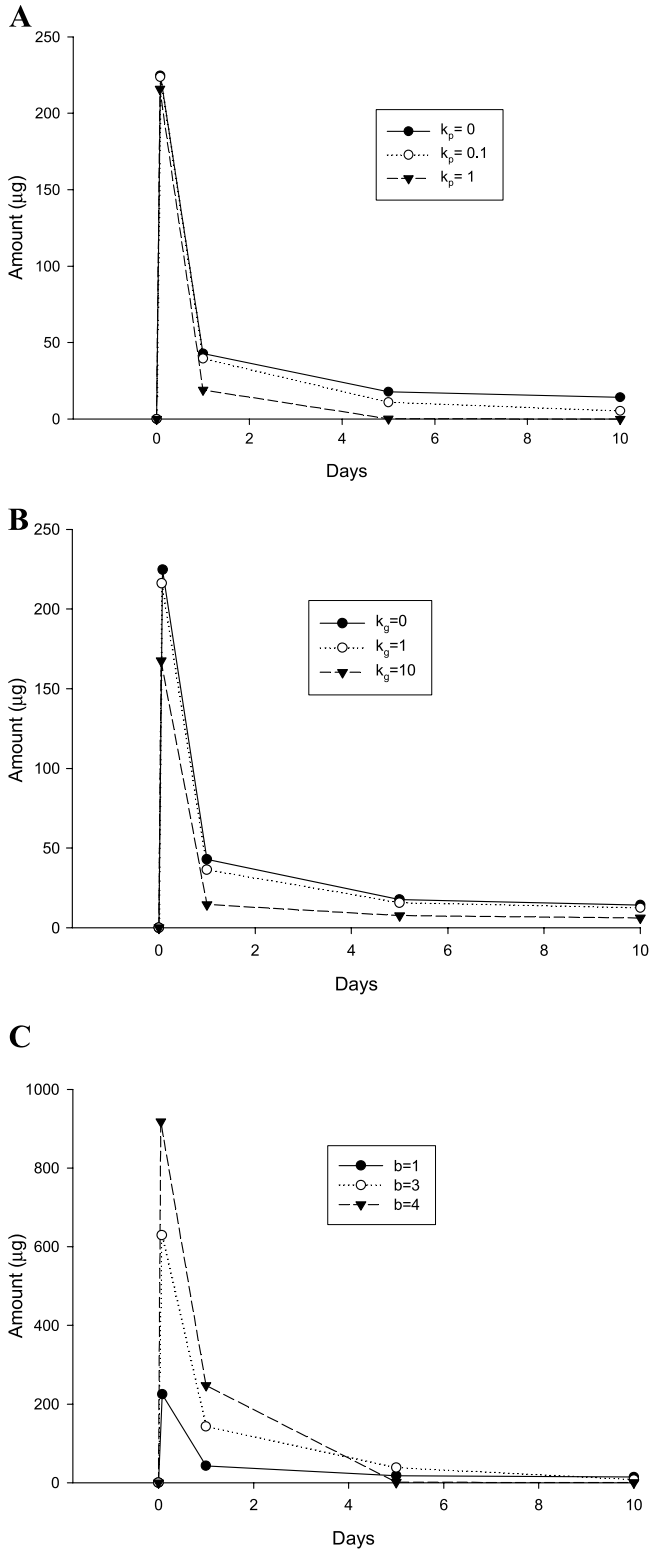

Fig. 9. Sensitivity analyses of model parameters affecting dipyridamole deposition within vessel/graft following perivascular administration. The profiles are simulated for drug deposition within explanted graft. (A) Effect of rate of drug loss (k_p) to the surroundings. (B) Effect of rate of drug loss (k_g) due to metabolism. (C) Effect of the surface area in terms of length b of perivascular application over the vessel/graft wall.

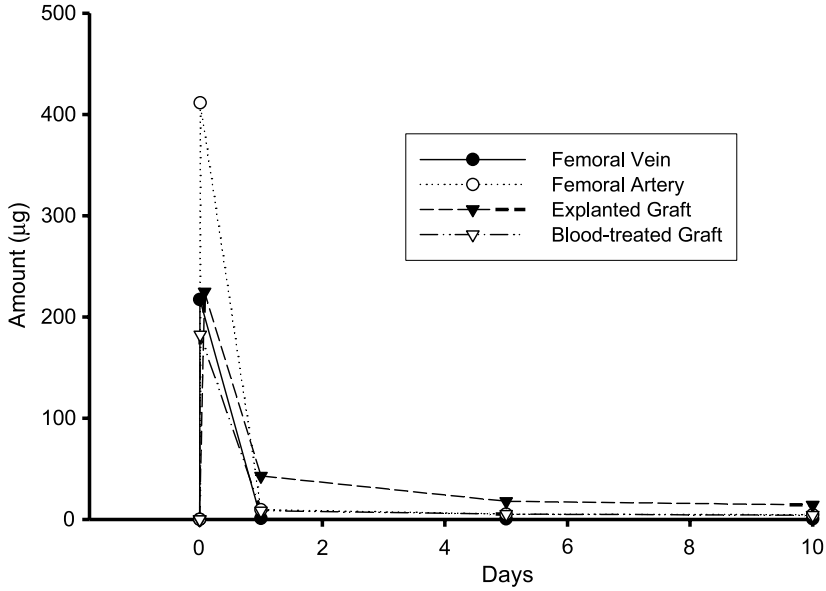

Fig. 10. Graft, arterial, and venous dipyridamole vessel/graft wall concentrations predicted by tissue pharmacokinetic model after administration of the same perivascular dose.

relative loss of drug within vessel walls due to metabolism as an assumed example (Fig. 9). These pharmacokinetic model simulations based on drug diffusivities and partition coefficients show that arteries, veins, and different types of grafts have varying local drug concentrations profiles within the vessel wall. The amount of drug deposited within arterial walls was higher than that within the venous walls after exposure to the same perivascular dose (Fig. 10).

For all the *in vitro* studies, mass balance showed at least 95% dose recovery for each experiment that was conducted. This was considered appropriate given the degree of variability inherent in the drug assay methods and tissue and fluid sampling where homogenous distribution is assumed in each type of tissue studied.

DISCUSSION

In this study, we determined the diffusivities of the hemodialysis vascular grafts and the surrounding native vessels for dipyridamole to evaluate the drug concentrations within the vascular walls when the drug is applied perivascularly. Because neointimal hyperplasia can develop in the graft and in the native vessels around the anastomoses, drug transports via the transgraft, transarterial, and transvenous pathways are all potentially important (Fig. 1). *In vitro* studies were performed to experimentally determine the diffusivities and partition coefficients for dipyridamole during perivascular administration of the drug. These *in vitro* results show that the diffusivities and partition coefficients through each pathway are unique. Therefore, the optimization of perivascular drug delivery requires the determination of these kinetic parameters via each pathway.

Although high permeance is desirable in many other applications, it might be limiting in the case of perivascular drug delivery, as illustrated in our predicted tissue pharmacokinetic model (Fig. 10). Because native veins were more permeable to dipyridamole and have lower partitioning coefficient, the concentration in the venous wall were lower than those in the arterial wall for the same amount of drug applied perivascularly (Fig. 10). High drug concentrations in the vascular wall are presumably desirable because that is the site of origin of the proliferating smooth muscle cells that participate in the formation of stenosis. High permeability with low partition coefficient would imply that the drug readily reaches the vascular lumen and is lost to the general circulation.

The diffusivities of dipyridamole through native arteries and veins were higher than that through the explanted graft, probably because the drug has to traverse the adventitial tissue layer surrounding the graft and the graft wall following perivascular drug administration. This situation might actually be advantageous via transgraft route because intimal hyperplasia occurs more frequently in the native vessels around the anastamosis than in the grafts (23). This adventitial tissue layer surrounding grafts acts an additional barrier to drug transport via transgraft pathway and would prevent any drug loss via this pathway. This would facilitate drug to be more available via transarterial or transvenous pathway, where hyperplasia actually originates.

A potential concern of perivascular drug delivery for the prevention of vascular stenosis is that the convective forces due to the pressure gradient between endovascular and perivascular space acting in the opposite direction resulting from the high hydrostatic pressure in the lumen might impede the transport of the drug from the perivascular side to the lumen. Our results, however, show that despite high hydrostatic pressure gradients (ΔP = 80 mmHg for grafts and arteries) in the opposite direction, drug diffusion into and across the vessels was not substantially impaired. The only exception was the native femoral vein, which was more permeable at high pressure gradient, ΔP = 10 mmHg. A potential explanation of this phenomenon is that veins are susceptible to expansion at high pressure gradients across its wall, which in turn may increase the wall porosity and

facilitate drug transport. For arteries and grafts, our data suggest that even against a steep hydrostatic pressure gradient (ΔP =80 mmHg), diffusion is the predominate force for drug transport. Similar observations have been reported for heparin transport across rat abdominal aorta wall (11). Although this seems to be true for grafts and arteries, the convective forces due to pressure gradient seem to enhance drug diffusion across veins. Investigation into the exact phenomenon causing this unique observation may further need to be explored.

The transport of the drug along any pathway is also dependent on its partitioning. Partition coefficients determine the degree of both specific and nonspecific binding of the drug in the vessel (13). Our tissue culture experiments show that there were no significant differences in the value of partition coefficients when the equilibration time was changed from 24 to 72 h (data not shown). Therefore, 24 h of equilibration was sufficient time to measure partition coefficient of dipyridamole within vessels and grafts. Although partition coefficients were determined by *in vitro* perfusion during nonequilibrium process, our results show that after 24 h of drug perfusion across vessels and buffer-treated graft was probably a good estimate of tissue culture experiments when equilibration had occurred with the perivascularly applied drug and vessel/graft wall. High partitioning in arteries suggest that they have high elastin content and enhance the degree of drug binding of hydrophobic drugs like paclitaxel (24). This might be the case for a hydrophobic drug like dipyridamole also with arteries, and because of this high binding, arteries have higher partition coefficient values compared to veins and grafts.

In order to predict drug concentration profiles in vessels derived from a perivascular depot, we developed a pharmacokinetic model based on the drug diffusivity within the perivascular tissue kinetics and partitioning data obtained from our *in vitro* experiments coupled with the known release kinetics of dipyridamole from the drug delivery system. Because dipyridamole is released from the drug delivery system over a period of weeks, it was not possible to use it within the *in vitro* experiments. However, given that the pharmacokinetics is linear, simulating the release profile of drug from the delivery system will give representative concentrations of the *in vivo* condition. Although this tissue pharmacokinetic model is validated on limited *in vivo* data, it provides reasonable predictions of local tissue concentrations of dipyridamole based on the experimentally derived kinetic data. These kinetic models can also be further explored for various drug delivery systems with different drug release rates. These results are helpful in predicting the perivascular dose to achieve therapeutic concentrations above IC_{50} values of vascular smooth muscle cells (which are 18.3 μg/mL for human aortic smooth muscle cells and 15.4 μg/mL for human venous smooth muscle cells (15,16). The sensitivity analyses shows that drug loss either potentially by metabolism or loss to the surroundings would decrease the amount of drug being deposited within the vessel or graft walls. These parameters are extremely important because it has been shown that the major route of drug transport was via extravascular capillaries rather than arterial route following perivascular administration (9). An increase in the surface area (b = 4) of perivascular application shows that the drug concentration would be higher initially but would have lesser drug available over time. A decrease in surface area (b = 1) of perivascular application, on the other hand, could cause higher availability of drug within vessel walls over a longer period (Fig. 9).

In summary, veins are more permeable than arteries, and arteries are more permeable than explanted grafts. Despite convective forces due to pressure gradients, the drug transport is dependent on drug diffusivity via grafts or arteries. On the other hand, the partitioning of dipyridamole into the arteries is highest followed by the veins and explanted graft. The different tissue pharmacokinetics based on diffusivities and partitioning of antiproliferative drugs into arteries and veins vessel wall need to be taken into account when designing drug dose and perivascular drug delivery systems to vascular tissue.

ACKNOWLEDGMENTS

The study is supported by the Research and Medical Services of the Department of Veterans Affairs, National Heart, Lung and Blood Institute (R01HL67646) and the Dialysis Research Foundation. The expanded polytetrafluoroethylene grafts were kindly donated by Bard Peripheral Vascular® (Tempe, AZ). The Utah Artificial Heart Institute manufactured the chamber used in the *in vitro* apparatus. Dr. Syed F. Mohammad and Mr. Craig D. Kamerath assisted in setting up the *in vitro* perfusion apparatus. Ms. Weiwei Zhu provided the release data of dipyridamole from thermosensitive polymer.

REFERENCES

1. P. Roy-Chaudhury, B. S. Kelly, M. A. Miller, A. Reaves, J. Armstrong, N. Nanayakkara, and S. C. Heffelfinger. Venous neointimal hyperplasia in polytetrafluoroethylene dialysis grafts. *Kidney Int.* **59**:2325–2334 (2001).
2. P. Roy-Chaudhury, B. S. Kelly, A. Narayana, P. Desai, M. Melhem, R. Munda, H. Duncan, and S. C. Heffelfinger. Hemodialysis vascular access dysfunction from basic biology to clinical intervention. *Adv. Renal Replace. Ther.* **9**:74–84 (2002).
3. P. Roy-Chaudhury, B. S. Kelly, J. Zhang, A. Narayana, P. Desai, M. Melham, H. Duncan, and S. C. Heffelfinger. Hemodialysis vascular access dysfunction: from pathophysiology to novel therapies. *Blood Purif.* **21**:99–110 (2003).
4. P. Roy-Chaudhury, R. Munda, J. Edwards, and B. S. Kelly. Hemodialysis vascular access dysfunction: from clinical stenosis to specific cell types. *J. Nephrol.* **16**:227 (2003).
5. L. M. Rainwater, G. Plate, P. Gloviczki, R. C. Bahn, L. H. Hollier, and M. P. Kaye. Morphologic quantitation of pseudointima and effects of antiplatelet drugs on vascular prostheses in goats. *Am. J. Surg.* **148**:195–202 (1984).
6. W. R. Brody, J. W. Brown, B. A. Reitz, D. L. Fry, and L. L. Michaelis. Effects of dipyridamol and methylprednisolone on intimal thickening in vein grafts. *J. Thorac. Cardiovasc. Surg.* **73**:601–604 (1977).
7. C. Chen, S. R. Hanson, and A. B. Lumsden. Boundary layer infusion of heparin prevents thrombosis and reduces neointimal hyperplasia in venous polytetrafluoroethylene grafts without systemic anticoagulation. *J. Vasc. Surg.* **22**:237–245; discussion 246–247 (1995).
8. T. Masaki, R. Rathi, G. Zentner, J. K. Leypoldt, S. F. Mohammad, G. L. Burns, L. Li, S. Zhuplatov, T. Chirananthavat, S. J. Kim, S. Kern, J. Holman, S. W. Kim, and A. K. Cheung. Inhibition of neointimal hyperplasia in vascular grafts by sustained perivascular delivery of paclitaxel. *Kidney Int.* **66**:2061–2069 (2004).

9. M. A. Lovich, L. Brown, and E. R. Edelman. Drug clearance and arterial uptake after local perivascular delivery to the rat carotid artery. *J. Am. Coll. Cardiol.* **29**:1645–1650 (1997).
10. E. R. Edelman, A. Nathan, M. Katada, J. Gates, and M. J. Karnovsky. Perivascular graft heparin delivery using biodegradable polymer wraps. *Biomaterials* **21**:2279–2286 (2000).
11. M. A. Lovich and E. R. Edelman. Mechanisms of transmural heparin transport in the rat abdominal aorta after local vascular delivery. *Circ. Res.* **77**:1143–1150 (1995).
12. C. W. Hwang, D. Wu, and E. R. Edelman. Physiological transport forces govern drug distribution for stent-based delivery. *Circulation* **104**:600–605 (2001).
13. C. W. Hwang, D. Wu, and E. R. Edelman. Impact of transport and drug properties on the local pharmacology of drug-eluting stents. *Int. J. Cardiovasc. Intervent.* **5**:7–12 (2003).
14. J. Himmelfarband and L. Couper. Dipyridamole inhibits PDGF- and bFGF-induced vascular smooth muscle cell proliferation. *Kidney Int.* **52**:1671–1677 (1997).
15. S. J. Kim, T. Masaki, J. K. Leypoldt, C. D. Kamerath, S. F. Mohammad, and A. K. Cheung. Arterial and venous smooth-muscle cells differ in their responses to antiproliferative drugs. *J. Lab. Clin. Med.* **144**:156–162 (2004).
16. T. Masaki, C. D. Kamerath, S. J. Kim, J. K. Leypoldt, S. F. Mohammad, and A. K. Cheung. *In vitro* pharmacological inhibition of human vascular smooth muscle cell proliferation for the prevention of hemodialysis vascular access stenosis. *Blood Purif.* **22**:307–312 (2004).
17. J. P. Singh, K. J. Rothfuss, T. R. Wiernicki, W. B. Lacefield, W. L. Kurtz, R. F. Brown, K. A. Brune, D. Bailey, and G. P. Dube. Dipyridamole directly inhibits vascular smooth muscle cell proliferation *in vitro* and *in vivo*: implications in the treatment of restenosis after angioplasty. *J. Am. Coll. Cardiol.* **23**:665–671 (1994).
18. J. A. Murillo Pulgarin, A. Alanon Molina, and P. Fernandez Lopez. Direct determination of dipyridamole in serum. *Anal. Biochem.* **245**:8–16 (1997).
19. I. Noh, M. A. Lovich, and E. R. Edelman. Mechanisms of heparin transport through expanded poly(tetrafluoroethylene) vascular grafts. *J. Biomed. Mater. Res.* **49**:112–119 (2000).
20. C. J. Creel, M. A. Lovich, and E. R. Edelman. Arterial paclitaxel distribution and deposition. *Circ. Res.* **86**:879–884 (2000).
21. W. Zhu, T. Masaki, Y. Bae, R. Rathi, A. K. Cheung, S. E. Kern, Development of a sustained-release system for perivascular delivery of dipyridamole. *J. Biomed. Mater. Res. B Appl. Biomater.* (2005) in press.
22. M. A. Lovich and E. R. Edelman. Computational simulations of local vascular heparin deposition and distribution. *Am. J. Physiol.* **271**:H2014–H2024 (1996).
23. R. Y. Kanterman, T. M. Vesely, T. K. Pilgram, B. W. Guy, D. W. Windus, and D. Picus. Dialysis access grafts: anatomic location of venous stenosis and results of angioplasty. *Radiology* **195**:135–139 (1995).
24. C. W. Hwang and E. R. Edelman. Arterial ultrastructure influences transport of locally delivered drugs. *Circ. Res.* **90**: 826–832 (2002).