

Arterial Ultrastructure Influences Transport of Locally Delivered Drugs

Chao-Wei Hwang, Elazer R. Edelman

Abstract—An incomplete understanding of the transport forces and local tissue structures that modulate drug distribution has hampered local pharmacotherapies in many organ systems. These issues are especially relevant to arteries, where stent-based delivery allows fine control of locally directed drug release. Local delivery produces tremendous drug concentration gradients and although these are in part derived from transport forces, differences in deposition from tissue to tissue imply that tissue ultrastructure also plays an important role. We measured the equilibrium drug uptake and the penetration and diffusivity of dextrans (a model hydrophilic drug similar to heparin) and albumin in orthogonal planes in arteries explanted from different vascular beds. We found significant variations in drug distribution with geometric orientation and arterial connective tissue content. Drug diffusivities parallel to the connective tissue sheaths were one to two orders of magnitude greater than across these sheaths. This diffusivity difference remained relatively constant for drugs up to 70 kDa before decreasing for larger drugs. Drugs also distributed better into elastic arteries, especially at lower molecular weights, with almost 66% greater transfer into the thoracic aorta than into the carotid artery. Arterial drug transport is thus highly anisotropic and dependent on arterial tissue content. The role of the local composition and geometric organization of arterial tissue in influencing vascular pharmacokinetics is likely to become a critical consideration for local vascular drug delivery. (*Circ Res.* 2002;90:826-832.)

Key Words: drug delivery ■ stent ■ ultrastructure ■ anisotropy ■ diffusion

Surgings interest in stent-based local delivery for vascular diseases has made clear the pressing need to quantify rigorously and understand drug transport and distribution in the arterial wall.¹⁻⁴ Drug distribution in the arterial wall is a function of the interplay between local physiological transport forces and the physical characteristics of the target tissue.^{5,6} Although transport forces have been implicated to be responsible for large local drug concentration variations stemming from local delivery,⁷ it is increasingly evident that their influence is at least matched by that of local arterial ultrastructure. Arterial distribution of paclitaxel, for instance, is critically dependent on nonspecific binding sites that vary spatially within a specific arterial segment and from artery to artery.^{8,9} Indeed, arteries in different vascular beds are composed of varying cellular and connective tissue content¹⁰ so that differential binding or transport within these tissue elements may produce dissimilar drug uptake among the different artery subtypes. Even within a single artery subtype, the cylindrical organization of cells and connective tissue layers may significantly enhance drug motion in one direction while retarding it in another.¹¹ Inhomogeneities in vascular drug deposition therefore reflect not only variations in local transport forces but also regional differences in arterial ultrastructure and, as a result, natural geometric variations in drug handling and distribution.

It remains unclear how to optimize the delivery of any particular drug because the difficulty of targeting to different artery subtypes is compounded by the wide range in size, charge, and molecular conformation of cardiovascular therapeutics.^{4,12} We therefore sought to examine local vascular pharmacokinetics by specifically considering how drugs with defined physical characteristics would distribute in tissues of defined ultrastructural characteristics. We measured the equilibrium drug uptake and the penetration and diffusivity of dextrans (4 to 250 kDa) and albumin in different orthogonal planes in bovine arteries from different vascular beds. Albumin is a carrier protein for hydrophobic drugs,⁹ and dextran, structurally similar to heparin and readily available in a range of molecular weights, has widely served as a model hydrophilic drug.¹²⁻¹⁴ We correlated transport properties of the drugs to connective tissue content, in large part elastin, and to geometric organization of the arteries and found significant variation in drug deposition and transport with tissue content and geometric orientation. Drugs distributed to a greater extent into more elastic compared with more muscular arteries, although this preferential distribution was less apparent above 70 kDa. Drug diffusivity was an order of magnitude greater in the direction parallel to the plane of the elastic sheaths (planar direction) than across (transmural

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From the Harvard-MIT Division of Health Sciences and Technology (C.-W.H., E.R.E.), Massachusetts Institute of Technology, Cambridge, Mass; Cardiovascular Division, Department of Medicine (E.R.E.), Brigham and Women's Hospital, Harvard Medical School, Boston, Mass.

Correspondence to Chao-Wei Hwang, Division of Health Sciences and Technology, Massachusetts Institute of Technology, Room 16-343, 77 Massachusetts Ave, Cambridge, MA 02139. E-mail cwhwang@mit.edu

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direction). Drug transport in the arterial wall is thus highly dependent on direction (ie, anisotropic) and on target artery elasticity. The natural dependence of vascular pharmacokinetics on local tissue structure and composition is likely to be a critical consideration for local delivery, especially as stent-based and other drug delivery strategies evolve toward more complex geometries.

Materials and Methods

Elastin Content and Drug Equilibrium Uptake

Bovine arteries from a range of vascular beds, obtained from an abattoir (Research 87, Marlborough, Mass), were cleaned by gently teasing away excess fat and fascia from the external aspect of the artery using fine jeweler's forceps and scissors. Cleaned arteries were then sectioned into 2- to 3-cm segments. Connective tissue in large arteries, primarily elastin, was isolated using the hot-alkali method,^{10,15} in which arterial segments, autoclaved at 125°C and 1.1 atm for 1.5 hours, were placed in 9 mL 0.1 N NaOH at 90°C to 95°C for 30 minutes, then in 10 mL of 8 mmol/L EDTA at 37°C for 1 hour, then in 10 mL of a 1:1 mixture of acetone and methanol overnight at 25°C, then transferred into 10 mL of PBS²⁺ (Sigma PBS; catalogue No. 1000-3, phosphate-buffered saline with 0.01 mmol/L calcium chloride and 0.1 mmol/L magnesium chloride added). Elastin content, defined as the ratio of elastin dry weight to artery wet weight, was determined after overnight lyophilization.

To determine arterial drug uptake, cleaned segments of the thoracic and abdominal aortas and internal carotid artery were weighed and immersed in 10-mL solutions of 2.5 mg/mL FITC-albumin (Sigma) or 4-, 10-, 20-, 70-, and 250-kDa FITC-dextran (Sigma) for 48 to 60 hours. It was determined that equilibrium was reached in <24 hours. Drug uptake into elastin was also measured for dextrans (0.5, 1, 2, and 3 mg/mL). The segments were transferred into sequential 10-mL baths of PBS²⁺ for 48 hours each until no drug was detectable in the eluent. Two to three sequential elution baths were generally required. Eluent fluorescence was measured with a spectrofluorimeter (Fluoroskan II, MTX Lab Systems) and converted to concentrations using a standard curve. Tissue drug concentration (C_{Tiss}) was computed using the relation

$$(1) \quad C_{Tiss} = \frac{\sum_i (C_i V_i)}{W \rho^{-1}}$$

where C_i and V_i are the elution drug concentration and bath volume for the i^{th} elution, W is the tissue wet weight, and ρ is the tissue density. The tissue density was determined by taking the ratio of the tissue wet weight to its volume, measured by immersing the specimen in PBS²⁺ in a 10-mL graduated cylinder and recording the change in fluid level. The equilibrium uptake coefficient is defined as $\kappa = C_{Tiss}/C_0$ where C_0 is the bulk-phase drug concentration.

Drug Infusion and Dispersion

Cleaned arterial segments 2 to 3 cm long were cannulated and inspected for leaks. The endothelial layer was denuded by gently passing a small wire loop through the arterial lumen.¹² Arteries were positioned in a perfusion apparatus, which recirculates PBS²⁺ with no transmural pressure.¹⁶ A solution of 20 mg/mL 20-kDa FITC-dextran was infused at 0.4 mL/h for 20 minutes by a syringe pump (Cole-Palmer 74900) through a blunt 5- μ L micropipette applied tightly against the external perivascular aspect of the arterial segment. The drug was allowed to diffuse for 15 to 30 minutes before the artery was snap-frozen in embedding medium (OCT, Tissue-Tek). Control arteries were handled in an identical fashion and snap-frozen immediately after infusion. Arteries were sectioned into 20- μ m transverse sections and imaged with a Nikon Optiphot-2 fluorescence microscope under a FITC filter set and a $\times 2$ objective. Autofluorescence was subtracted,¹⁷ and drug dispersion was mea-

sured by tracking the advancing drug front of a threshold intensity to a resolution of 8 μ m.

Drug Penetration Depth

To measure transmural penetration depth, large arterial segments were immersed in FITC-dextran solutions (10 mg/mL) of various molecular weights for 15 minutes. This time interval was found to be sufficient to generate an adequate signal-to-noise fluorescence intensity ratio without drug entering the artery from one face diffusing deep enough to interfere with drug entering from the opposite face. Transmural penetration depths from either the luminal face or the perivascular face can thus be determined by imaging near the luminal or perivascular sides, respectively, of vessels transversely sectioned into 20- μ m slices. To measure planar penetration depth, a special planar diffusion cell was constructed by clamping arterial segments en face between two glass slides separated by spacers. The spacer width (≈ 1 mm) was adjusted for a tight seal between the specimen and the slide without crushing the tissue. Drug solutions were infused into the space between the slides and allowed to diffuse into the artery for 15 minutes. Planar penetration depth was determined from fluorescence images of arterial specimens cryosectioned en face into 20- μ m slices. To minimize edge effects, only the middle portion of each sample was imaged. Penetration depths corresponding to each fluorescence intensity were obtained from the images using MATLAB (Mathworks). The ratio of diffusivity in the two orthogonal planes is related to the ratio of penetration depths by $D_p/D_T = L^2_p/L^2_T$, where D is diffusivity, L is penetration depth, and P and T indicate the planar and transmural directions, respectively.¹⁸

Measurements of Transmural and Planar Diffusion

Planar and transmural diffusivities were measured using diffusion cells. Planar diffusivities were measured by mounting the arterial specimen into a planar diffusion cell (described in Drug Penetration Depth) and infusing FITC-albumin or FITC-dextran solutions (2.5 mg/mL) into the space between the slides. After a defined diffusion time, specimens were then cryosectioned en face into 20- μ m slices and transferred in groups of five into a 5-mL PBS²⁺ bath for 48 hours. Only the concentrations of slices at least 100 μ m from the glass slide were considered. For transmural diffusivity, cleaned arteries with denuded endothelium were spliced open longitudinally and clamped in a standard diffusion cell with either the luminal or adventitial side facing drug. A standard diffusion cell consists of two compartments, one containing drug and the other containing PBS²⁺, separated by an artery lying en face. The artery is thus exposed only to drug on one face and only to buffer on the other. After a defined diffusion time, drug was eluted out of the artery into a 5-mL PBS²⁺ bath for 48 hours. Eluent drug content was measured using a spectrofluorimeter (Fluorolog 1681, SPEX). The diffusion time was 20 minutes when drug molecular mass was 70 kDa or less, and 30 minutes when larger compounds were examined.

The structure of the arterial wall is highly heterogeneous, and it is clear that different tissue layers will have different individual diffusivities. However, because the heterogeneity of the arterial wall is highly regular (ie, the cylindrical bands of connective tissue and smooth muscle cells alternate on a regular basis), it is convenient to use a lumped effective diffusivity parameter to characterize bulk drug transport properties in the arterial wall. Lumped effective diffusivities can be calculated from the measured drug mass M in tissue, using the early time solution to the diffusion equation

$$(2) \quad D = \frac{\pi}{4} \cdot \left(\frac{M}{AC_0 \kappa \sqrt{t}} \right)^2$$

where t is the time, A is the artery area exposed to drug, κ is the partitioning coefficient, and C_0 is the drug source concentration.¹⁸ This expression has often been used to measure drug diffusivities in gels¹⁹ and is valid when the experiment duration is much shorter than tissue equilibration time t_{eq} , as was the case here. As a rough guide, the order of magnitude relation $t_{eq} \sim L^2/D$ yields an estimated t_{eq} of 3

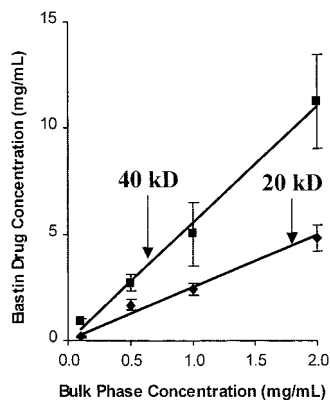


Figure 1. Equilibrium uptake of 20-kDa (◆) and 40-kDa (■) dextrans into isolated arterial elastin as a function of bulk-phase drug concentration. The ratio of the elastin drug concentration and the bulk-phase concentration is the equilibrium drug uptake coefficient.

hours in the transmural and 75 hours in the circumferential planes. We found that our diffusion time of 20 to 30 minutes provides an acceptable signal-to-noise ratio while satisfying $t \ll t_{eq}$.

Results

Artery-Dependent Drug Transfer

The equilibrium drug uptake (κ) into isolated elastin, a major component of arterial connective tissue, was compared with administered concentration and was for each dextran compound greater than unity, suggesting a high degree of nonspecific binding. This binding increased with dextran size such that for 40-kDa dextrans, there was a 5.5-fold increase above bulk-phase concentrations (Figure 1). The multicomponent structure of the artery thus hints that drug transfer might be dependent on the relative proportion of elastin versus smooth muscle cell content. Indeed, our measurements of κ into whole arterial media of different vascular beds correlated with arterial elastin content with almost 66% greater drug transfer into the thoracic aorta than into the internal carotid artery but nearly identical drug transfer to the thoracic and abdominal aortas for all molecular weights (Figure 2).

Dispersion of Infused Drug

Drug distribution is dependent not only on the amount and type of the various arterial component structures but also on the three-dimensional geometric arrangement of these components within the blood vessel wall. We visualized the dispersion of 20-kDa FITC-dextran in the arterial media subsequent to high-dose rapid infusion by tracking the evolution of drug distribution relative to natural geometric directions in the artery. The circumferential extent of drug distribution was determined by measuring the distance around the circumference of the artery where drug concentration exceeded a threshold value. We found that rapid drug dispersal decreased the circumferential extent of drug distribution to $75.3 \pm 4.9\%$ ($n=5$) of the initial distribution within 15 minutes and after 30 minutes further decreased to $68.2 \pm 2.4\%$ ($n=4$) of the initial

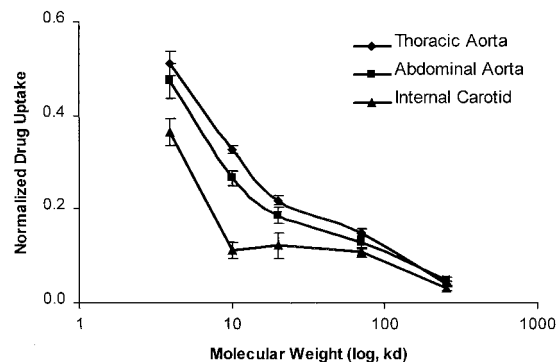


Figure 2. Uptake of dextrans normalized to administered drug concentrations in the thoracic aorta (◆), abdominal aorta (■), and internal carotid artery (▲) as a function of molecular mass (4 to 250 kDa).

distribution. The sharpness of this decrease is incompatible with isotropic diffusion. When a conservative estimate of $10 \mu\text{m}^2/\text{s}$ for the effective diffusivity of 20-kDa FITC-dextran in carotid arteries was used, an isotropic diffusion model predicted a slower decrease, to 96% and 94% of initial drug extent after 15 and 30 minutes, respectively, differing significantly from our observations ($P < 0.05$ at both 15 minutes and 30 minutes). Indeed, although only an estimate, the model matched observations only when $D \approx 200 \mu\text{m}^2/\text{s}$, far beyond typical values for transmural diffusivity for 10- to 40-kDa FITC-dextran,¹² suggesting anisotropic arterial diffusion. Rapid circumferential transport may result in much faster drug coverage of the endoluminal aspect of the media than the adventitial aspect (Figure 3).

Drug Penetration Depths

The cylindrical arrangement of tissue elements in the arterial wall suggests that the asymmetric drug dispersion we observed might arise from differential drug transport in the planar versus the transmural directions. To isolate this effect, we measured relative drug penetration depths while restraining predominant drug motion to either the transmural direction or the planar direction. After 15 minutes of free diffusion in the bovine aorta, planar penetration surpassed transmural direction by 369% and 153% for 4-kDa and 20-kDa FITC-dextran ($n=3$), respectively, corresponding to planar-to-transmural diffusivity ratios of 21.05 and 6.40. Transport in the carotid artery was similarly anisotropic, with planar penetration surpassing transmural penetration by 231% for 20-kDa drug ($n=3$), corresponding to a planar to transmural diffusivity ratio of 10.99 (Figure 4).

Bulk Diffusion Measurements

Effective planar and transmural diffusivities in the carotid artery were measured directly from the mass of drug transferred into the arterial wall ($n=7$) by using the early-time solution of the diffusion equation. Dextran planar and transmural diffusivities decreased with increasing molecular mass, gradually below 150 kDa and sharply

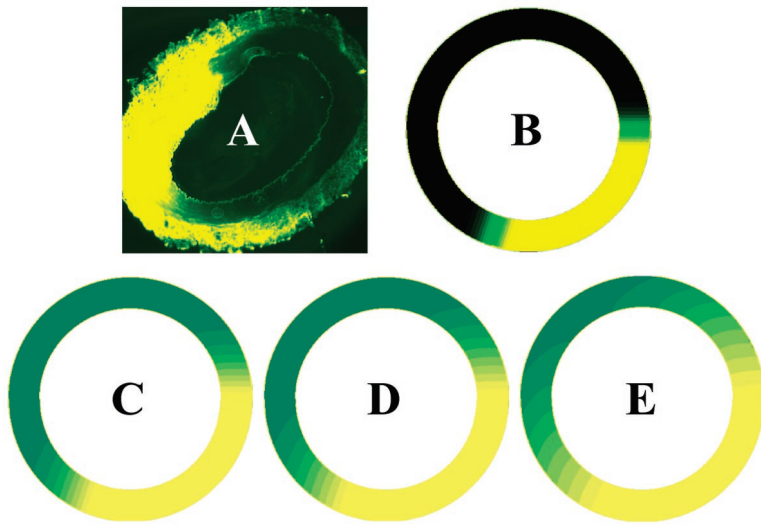


Figure 3. Observed drug extent in the media (A) at 30 minutes was comparable to the predicted distribution of an anisotropic model (B). C through E, Simulated spread of infused drug at 2, 4, and 12 hours using an anisotropic model shows more rapid drug coverage of the endoluminal aspect of the arterial wall than the adventitial aspect. The color scheme in C through E was rescaled to more clearly display lower drug concentrations.

beyond (Figure 5). It is thus apparent that mathematical relationships which assume a simple molecular conformation and a homogeneous solvent, such as the Stokes-Einstein equation,²⁰ although often used to estimate diffusivities of small molecules, are less useful in describing transport processes of complex large molecules in highly intricate biologic tissues. Transmural diffusivities were significantly less than planar diffusivities for molecular masses up to 150 kDa ($P < 0.05$). There was little differ-

ence between transmural diffusivities as measured from the luminal or adventitial aspect of the arterial wall. Transport was most anisotropic for drugs of lower molecular masses with planar diffusivity exceeding transmural diffusivity by 35- to 45-fold for 10- to 70-kDa drug and 12-fold for 150-kDa drug. Planar was identical to transmural diffusivity for 250-kDa drug. Albumin diffusivity is similarly anisotropic. Although our measurements for transmural albumin diffusivity are comparable to those made by others,²¹ planar diffusivity exceeded those values by a factor of 12.5 ($n = 7$) (Figure 5).

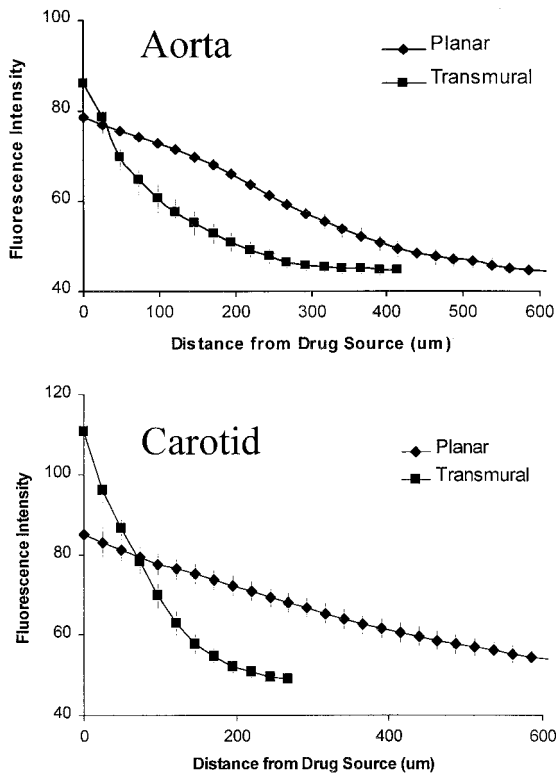


Figure 4. Penetration of 20-kDa FITC-dextran in the abdominal aorta and internal carotid artery in the plane parallel to the connective tissue elastin sheaths (planar, \blacklozenge) and across the planes of the sheaths (transmural, \blacksquare) measured in absolute fluorescence intensities.

Discussion

Local Influences on Drug Transport

Arterial ultrastructure and geometry dictate the transport of locally delivered drugs. Our data suggest that the motion of drug molecules through tissues is direction-dependent, such that transport rates differ widely in different planes, and deposition is exquisitely sensitive to ultrastructure and tissue content. Drugs will partition into one arterial component (eg, connective tissue elastin) at the expense of another (eg, smooth muscle cells), so that, for example, elastic arteries

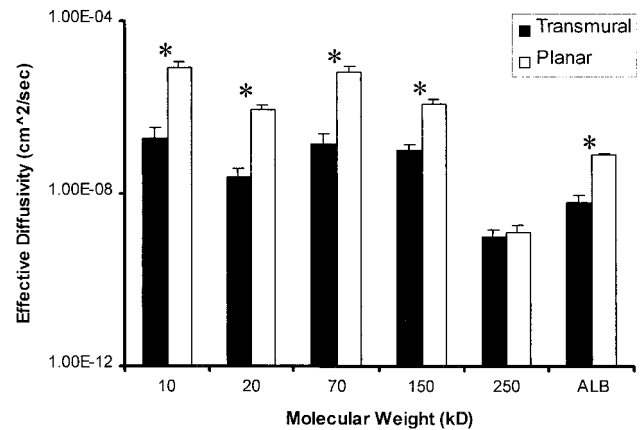


Figure 5. Effective diffusivity of albumin (ALB) and dextrans as a function of molecular weight in the planar (white bars) and transmural (black bars) directions in the internal carotid artery. *Planar and transmural diffusivities are statistically different ($P < 0.05$).

will naturally take up more drug than muscular arteries. These findings have profound implications for vascular drug delivery because maintaining an appropriate drug distribution in the arterial wall on the scale of microns is requisite for effective delivery.²² Unfortunately, tremendous drug concentration gradients are invariably established by transport forces such that drug concentrations in adjacent cells can differ significantly.⁷ Our data suggest that these gradients might be further amplified by the heterogeneous arterial drug uptake characteristics. Thus, local tissue ultrastructure and the concentrations they enforce on the artery at a microscopic scale become important considerations in the optimization of vascular drug delivery.

Vascular Drug Transport Is Dependent on Geometric Direction

Most studies of arterial transport have focused on movement of the drug in the transmural direction to the exclusion of others.^{12,23} This reflects in part the desire to understand an assumed need to drive drugs across the vessel wall as well as the presumption that arterial transport is isotropic, with flux in one direction mirroring flux throughout the vessel. We have now shown the latter to be faulty and that diffusivity in the planar direction exceeds transmural diffusivity by at least one order of magnitude, for disparate compounds that span a wide range of molecular weights. The highest anisotropy occurred for drugs up to 70 kDa, with steric effects dampening the anisotropy for drugs beyond this range. Models of arterial wall drug distribution based on the prevailing assumption of isotropic arterial transport thus might overestimate circumferential drug concentration gradients because of the actuality of faster planar diffusivities.

The multilaminar structure and differential permeability of the arterial wall components conform to the notion of anisotropic arterial diffusion. Although individual tissue layers might be isotropic, a composite alternating between layers of increased and decreased permeability impedes transmural transport while not interfering with transport within the plane of the material. Indeed, Winlove and colleagues measured the decreased permeability of the elastic lamina to macromolecules,^{24,25} showing these barriers to pose significant resistance to gene transfer vectors.²⁶ Penn et al²⁷ specifically examined the resistance of the internal elastic lamina to the transport of horseradish peroxidase in the context of drug delivery. Although the internal elastic lamina certainly plays a large role in transmural resistance, our data showed that diffusive anisotropy persisted regardless of whether transmural diffusivity is measured from the luminal or the adventitial side. This suggests that deeper elastic layers can also affect transport.

Arterial Wall Components Influence Drug Deposition

Our results are consistent with theoretical studies by Weinbaum et al,¹¹ which postulate anisotropic transport in the arterial wall due to its multilaminar structure, but we also show that the component tissue types of the arterial wall may themselves influence drug distribution. The isolated elastin component has a drug uptake coefficient greater than unity,

indicating a degree of nonspecific binding far beyond that of the arterial wall as a whole. Delivered drugs will thus preferentially localize themselves within the elastin sheaths, creating locally high concentrations. Fluorescence microscopy of arterial wall drug deposition shows dramatic intensity spikes at elastin sheath positions,²⁸ even after compensating for autofluorescence.¹⁷ Others have also documented significant interactions of albumin, lipoproteins, and calcium salts with elastin.^{29,30}

Such differential binding has several important consequences. First, differential uptake into individual arterial layers causes elastin-rich regions deep in the artery to act as drug sinks and sources, evolving from one to the other in time. Second, the dependence of drug uptake on molecular weight is affected. Equilibrium uptake decreases with increasing molecular weight, but the decrease is slower in arteries with higher elastin content, possibly because nonspecific binding into elastin compensates for the increasing steric hindrance. Finally, the amount of drug transferred to the arterial wall by local delivery is dependent on the proportion of elastin content. Drugs with molecular masses ranging from 4 to 70 kDa distributed significantly better into elastic arteries than into muscular arteries. As such, arterial tissue content must be a consideration in dosing, especially when applying doses used in one artery to another artery of different ultrastructural characteristics.

Implications for Hydrophobic Drugs

Arterial transport of hydrophobic drugs, such as paclitaxel, is complicated by their numerous interactions with fixed tissue elements and diffusible serum proteins. Hydrophobic drugs partition highly into arterial tissue, resulting in arterial drug concentrations in some cases more than an order of magnitude above applied levels.⁸ In particular, Creel et al⁸ found paclitaxel to be deposited more heavily in the intima and adventitia, with a gradient extending inward into the media from both sides, presumably because of a heterogeneous distribution of fixed binding sites. Our data imply that interplay between drugs and naturally hydrophobic arterial tissue layers (such as elastin) might contribute to and, especially in elastic arteries, enhance this baseline heterogeneity, potentially resulting in concentration spikes corresponding to the elastic laminae. Hydrophobic drug interactions with abundant serum proteins further complicate hydrophobic drug transport processes. Using paclitaxel as a model, Lovich et al⁹ found that serum proteins, in large part albumin, can enhance hydrophobic drug solubility while at the same time slowing drug transfer into the arterial wall at the stent-artery interface. Once within the arterial wall, the relative affinity of the drug to the serum protein versus the fixed tissue binding sites becomes a determinant of transport.⁹ Because our results show that albumin exhibits preferential planar to transmural arterial diffusion, drugs with tighter association to serum albumin may well diffuse in an anisotropic manner within the arterial media. Thus, hydrophobic drug transport reflects the intricate interplay between drug diffusion, partitioning, and binding events to both fixed and diffusible binding sites. Each of these issues needs to be

considered to predict optimal dosing in hydrophobic drug delivery systems.

Transport in Pathological Arteries

Vascular disease can significantly increase the complexity of arterial drug transport and distribution. Although animal models of neointimal, lipid-laden, and stent-induced pathologies have long existed,^{31–33} the resulting lesions are highly variable and, as a result, drug transport through these lesions has remained difficult to assess experimentally. Nevertheless, it is possible from studies of nondiseased arteries to infer some general transport properties one might expect to find in diseased arteries given specific lesion characteristics. The hyperplastic neointimal layer, for instance, is typically composed of smooth muscle cells without distinct geometric orientations.³⁴ Because the diffusive anisotropy in the arterial wall is a product of the geometric orientation of smooth muscle cells and the regular layering of tissues of differing permeabilities, one might expect to find isotropic drug transport in the neointima. Furthermore, because of the absence of the relatively impermeable elastin barriers, neointimal drug diffusivity might be closer to medial diffusivity in the planar direction (which also lacks a transport barrier) than in the transmural direction. Compared with the neointima, lipid-laden atherosclerotic plaques might be expected to favor more highly hydrophobic drugs, and their fibrous caps might exclude drugs of higher molecular weights. Finally, stented arteries with highly compressed media may actually exhibit even higher diffusive anisotropy than native arteries because medial compression tightly packs arterial tissue layers together,³⁵ further limiting transmural transport compared with planar transport. Because diseased arteries are the ultimate target of vascular drug delivery, the effects of pathological arterial states on tissue structure and drug transport clearly merit further experimental study.

Summary

Local drug distribution is influenced by ultrastructure through both the geometric organization and the variable avidity for drug uptake of the component arterial tissues. Drugs do not distribute evenly throughout blood vessels. Drug transport is highly dependent on direction and plane of the tissue, and their deposition and uptake are determined by tissue contents. Elastin in particular binds drugs with great affinity, leading to greater drug entry into and local pooling within highly elastic arteries. Optimization of local delivery thus requires a careful consideration of the underlying arterial ultrastructure and resulting effects on the transport of delivered drug. The potential of local drug delivery is tremendous once adequate targeting is achieved, and thus a rational approach to the design of local delivery devices will require a careful consideration of the interplay of drug with arterial tissue organization and composition.

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