Transport of $^{125}$I-Albumin Across Normal and Deendothelialized Rabbit Thoracic Aorta In Vivo

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Transmural concentration profiles of $^{125}$I-albumin in vivo were measured across the normal and balloon catheter-deendothelialized rabbit descending thoracic aorta as a function of time following intravenous injection. A tracer was injected 5 or 60 minutes after deendothelialization, and the animals were sacrificed after circulation times of 10, 30, or 60 minutes. The aorta was immediately excised and frozen flat between glass slides. Samples were serially sectioned parallel to the intimal surface in a refrigerated microtome, washed with trichloroacetic acid (TCA), and counted. Relative tissue concentration profiles of TCA-precipitable radioactivity from the media of control animals showed entry from both luminal and adventitial sides, as previously found with conscious normal rabbits, but spatial gradients at both luminal and medial-adventitial borders were less steep. Relative concentration levels in balloonned animals were 10- to 40-fold higher than in controls, and the profiles were flatter. Uptake rates at equivalent circulation times were greater in experiments initiated 60 minutes, as compared with 5 minutes, after deendothelialization, suggesting that progressive medial edema may have occurred following balloon injury. These results show that the intact endothelium is the dominant mass transfer resistance for $^{125}$I-albumin transport across the aortic wall. The data also suggest that the incomplete monolayer of platelets adherent to the subendothelium after balloon deendothelialization is not a substantial resistance to transport, as compared to that of the media, and that convection plays a more important role than diffusion for $^{125}$I-albumin transport across the deendothelialized aortic wall. (Arteriosclerosis 4:283-291, May/June 1984)

The transport and metabolism of plasma proteins in the arterial wall may be important in atherogenesis. The endothelium is thought to play an important role in limiting rates of uptake in the arterial wall. Transendothelial passage of plasma solutes depends upon molecular size,1 and the mechanisms available to larger solutes are limited to vesicular transport and/or transport through intercellular junctions. Previous studies of macromolecular transport across injured arterial endothelium and into deendothelialized vessels, including in vivo measurements with albumin-bound Evans blue dye2,3 and $^3$H-cholesterol4, and in vitro5,6 and in situ studies with Evans blue and $^{125}$I-albumin, suggest that the endothelium represents the primary resistance to transport across the arterial wall. Using measurements of the distribution of labeled albumin5 and low-density lipoprotein6 across the thoracic aorta of normal conscious rabbits in vivo, together with approximate mass transfer calculations, we previously estimated that the mass transfer resistance associated with the intimal endothelium is about ten times that associated with the aortic media.

The objective of the present study was to determine quantitatively the effects of endothelial removal on albumin uptake in the arterial wall. We measured concentration profiles of radiiodinated albumin across the descending thoracic aortic wall as a function of time following intravenous injection into anesthetized rabbits, with and without balloon catheter-deendothelialization of the thoracic aorta. Measurements were made with the frozen tissue serial sectioning technique used in our earlier studies.5,6
A second objective of this study was to determine if the incomplete platelet monolayer that forms on the deendothelialized aortic wall functions as a significant mass transfer barrier. To this end, uptake experiments were initiated either 5 minutes or 60 minutes after balloon-deendothelialization, at which times the extents of platelet surface coverage were substantially different.

Methods

Animal Preparation

Twenty-eight New Zealand white male rabbits weighing 2.8 to 3.5 kg each were anesthetized by intravenous injection of 1.5–2.5 ml of a 10% (wt/vol) solution of sodium pentobarbital (Somnethol, J.A. Webster Incorporated, North Billerica, Massachusetts) delivered over 20 to 30 minutes. Anesthesia was supplemented with ether as needed. A femoral artery was exposed, isolated, and ligated distally. A 4F Fogarty arterial embolectomy balloon catheter (Edwards Laboratories, Santa Ana, California) was inserted and pushed to the level of the diaphragm, never inflated. The catheter was never pushed past the level of the diaphragm and never inflated.

Injection of Labeled Albumin

Rabbit serum albumin (4X crystallized, ICN Pharmaceuticals, Incorporated, Life Sciences Group, Cleveland, Ohio) was iodinated with Na 125I (17 Ci/mg in 0.1N NaOH, New England Nuclear, Boston, Massachusetts) by using the iodine monochloride method9 with a 1:1 molar ratio of albumin to iodine. Labeling efficiencies were about 70% as determined by precipitation of protein-bound radioactivity with 10% (wt/vol) trichloroacetic acid (TCA). Nonprotein-bound radioactivity was removed by dialysis against 2000 volumes of 0.01% (wt/vol) disodium ethylenediaminetetraacetate (EDTA) in isotonic saline. Protein-bound radioactivity in the injectates averaged 99.4% of the total radioactivity. Homogeneity was verified by chromatography on Sephadex G-200 and electrophoresis on cellulose acetate.9

At 5 or 60 minutes after deendothelialization, 125I-albumin (2 mCi in 1 ml) was injected through a filter (Millex Disposable Filter Unit, Sterile 0.22 μm, Millipore Corporation, Bedford, Massachusetts). The filter was flushed before use with 1 ml isotonic saline and after 125I-albumin injection with 5 ml isotonic saline. The latter rinse was also injected. Blood samples for plasma radioactivity determination were taken from the femoral cannula 5 minutes after injection and at specified times thereafter. Each sample was collected in a glass tube containing 10.5 mg disodium EDTA (Vacutainer Tube, M32040, Becton, Dickinson and Company, Rutherford, New Jersey), spun at 2400 rpm at 4°C for 20 minutes, and the plasma was pipetted into a 3 ml siliconized test tube for radioactivity assay.

Animal Sacrifice and Excision of Aorta

At 10, 30, or 60 minutes following the injection of labeled albumin, the animals were sacrificed with an overdose of sodium pentobarbital. The thoracic cavity was opened, and the descending thoracic aorta was exposed, excised, cut open longitudinally, rinsed sparingly with isotonic saline, and quickly frozen between glass slides (Cryokwik, IEC, Damon/IEC Division, Needham Heights, Massachusetts). The time between death and freezing was less than 6 minutes, and the tissue was wrapped in aluminum foil and stored at −20°C for 1 to 3 days before further processing.

Sectioning of Aortic Samples

Samples of the frozen aorta between pairs of intercostal arteries (about 0.5 cm2 luminal surface area) were serially sectioned in a refrigerated microtome (No. 50-ABN Rust-Proof Rotary Microtome, Lipshaw Manufacturing Company, Detroit, Michigan or No. 6501 Harris Model WRC Wide Range Microtome Cryostat, Harris Manufacturing Company Incorporated, North Billerica, Massachusetts) at a temperature of −20°C using a slight modification13 of the procedure previously described.9 Tissue sectioning proceeded from the adventitia along planes parallel to the luminal surface at a thickness of 10 μm per slice. Adventitial slices were discarded. Each medial slice was picked from the microtome knife with absorbing paper wrapped around cold forceps. Both tissue slice and paper were deposited at the bottom of a 3 ml siliconized test tube. The tissue sample perimeter of the innermost slice was traced on a precalibrated plastic sheet. The area of the frozen tissue was determined by weighing the tracing, and the volume of each slice calculated. The total thickness (L) of frozen tissue between the luminal and medial-adventitial borders was estimated to within ±20 μm. In a separate study,14 we have shown that the thickness of this relaxed frozen tissue is 1.72 times greater than its actual thickness in vivo.

Radioactivity Assay

To each tube containing a tissue slice, we added 0.025 ml of 6% bovine serum albumin (wt/vol) and 2 ml of 10% (wt/vol) TCA. The tubes were agitated gently and stored at 4°C for 30 minutes. They were then spun at 2400 rpm at 4°C for 20 minutes, and the supernatant was carefully removed by pipette and discarded. The TCA wash was repeated once, and the slices were counted for at least 10 minutes in a
well-type gamma scintillation counter (Model 5024, Packard Instrument Company, Downers Grove, Illinois) at a counting efficiency of 75%. A similar procedure was used with injectate and plasma samples. All slices had counting rates in excess of 20 counts/min (CPM) above background (30–80 CPM).

Results

The initial TCA-precipitable $^{125}$I-albumin concentration in plasma ($C_{Po}$) ranged from about 1 to $3 \times 10^7$ counts/min per ml plasma. The plasma concentration decreased linearly with time to 86% of $C_{Po}$ after 60 minutes circulation in control animals, in accord with our previous observations and to 80% of $C_{Po}$ after 60 minutes in the ballooned animals. The fraction of total plasma radioactivity that was not TCA-precipitable increased from about 0.006 to 0.01 after 60 minutes circulation in control animals and remained constant at 0.006 in ballooned animals.

Concentration profiles of TCA-nonprecipitable tissue radioactivity were relatively flat across the media, and the average concentration increased from the 10- to 60-minute tracer circulation times in both control and ballooned animals. The TCA-nonprecipitable radioactivity accounted for about 30% of the total tissue radioactivity in the control animals and between 5% and 10% in the ballooned animals. The ratio of the TCA-nonprecipitable radioactivity concentration in tissue to the initial TCA-nonprecipitable radioactivity concentration in plasma was about 0.1 and 0.4 for the 10- and 60-minute circulation experiments initiated 5 minutes after sham-ballooning in control animals, respectively, and about 0.5 and 3.0 for the comparable experiments in ballooned animals. The ratios for control animals were similar to those obtained previously. The ratios for ballooned animals suggest an even more rapid entry of TCA-nonprecipitable radioactivity into the arterial wall in the absence of endothelium and the possible generation of TCA-nonprecipitable radioactivity within the media during the 60-minute circulation experiments.

Each transport experiment is designated by two digits. The first indicates the elapsed time in minutes between ballooning (or sham-ballooning) of the animal and injection of $^{125}$I-albumin, and the second indicates the total elapsed time from ballooning to sacrifice of the animal. The difference between the two digits is the time interval for tracer uptake. For example, 5–15 min denotes a 10-minute uptake experiment initiated 5 minutes after balloon catheter deendothelialization (or sham-ballooning in controls).

Figure 1 shows transmural profiles of the relative tissue concentration ($C_T/C_{Po}$) of TCA-precipitable radioactivity across six different samples of the aortic wall of a single control rabbit in a 10-minute uptake experiment initiated 5 minutes after sham balloon-deendothelialization (5–15 minutes, Run 3). $C_T$ is the concentration of TCA-precipitable tissue radioactivity (counts/min/cm$^2$ wet tissue); $C_{Po}$ is the initial measurement of TCA-precipitable radioactivity in plasma (counts/min/cm$^2$ plasma); $x$ is the distance from the intimal surface to the midpoint of the tissue slice; and $L$ is the distance between the intimal surface and the medial-adventitial border as determined in the relaxed frozen state (average $L = 230 \mu m$). Each open symbol represents the relative concentration at the midpoint of a single tissue slice. Each solid circle represents a local average of data from spatially congruent (in x/L) tissue slices from different samples of the same aorta.

Figure 1. Transmural profiles of relative tissue concentration ($C_T/C_{Po}$) of TCA-precipitable radioactivity across six different samples of the aortic wall of a single control rabbit in a 10-minute uptake experiment initiated 5 minutes after sham balloon-deendothelialization (5–15 minutes, Run 3). $C_T$ is the concentration of TCA-precipitable tissue radioactivity (counts/min/cm$^2$ wet tissue); $C_{Po}$ is the initial measurement of TCA-precipitable radioactivity in plasma (counts/min/cm$^2$ plasma); $x$ is the distance from the intimal surface to the midpoint of the tissue slice; and $L$ is the distance between the intimal surface and the medial-adventitial border as determined in the relaxed frozen state (average $L = 230 \mu m$). Each open symbol represents the relative concentration at the midpoint of a single tissue slice. Each solid circle represents a local average of data from spatially congruent (in x/L) tissue slices from different samples of the same aorta.

Figure 1 responds to a single sample obtained from the tissue between each of the six pairs of intercostal arteries. Profiles from individual samples were reasonably consistent. $C_T/C_{Po}$ ranged from about 0.0005 to 0.0015 near the middle of the media. It was about fivefold higher near the luminal surface ($x/L = 0$) and was at an intermediate level near the medial-adventitial border ($x/L = 1$). No systematic variation in relative concentration profiles along the length of the descending thoracic aorta was observed in any experiment.

The solid circles in Figure 1 represent averages of data from tissue slices spatially congruent in x/L in the same aorta. The average profiles for each rabbit are shown in Figure 2 for control animals and in Figures 3 and 4 for ballooned animals. The error bars are omitted for clarity. The coefficient of variation of $C_T/C_{Po}$ averaged about 0.4 across most of the media in all experiments. Figure 5 shows a comparison of the grand average profiles for each group of experiments.
In control animals, the concentration level in the middle of the media increased continuously with time. Concentration gradients near both the luminal and the medial-adventitial borders were observed in the 5–15 and 5–35 minute control experiments, thereby suggesting the entry of solute into the media from both the lumen and the adventitia. In balloon-deendothelialized animals, $C_v/C_{p0}$ in the media increased with time and was about 10- to 40-fold greater than that of control animals with corresponding tracer circulation times. Concentration profiles across the media of ballooned animals were flatter than the control profiles, the gradients at the boundaries of the media being, for the most part, eliminated. The concentration was usually higher near the luminal border than near the medial-adventitial border in all the 5–15 and 5–35 minute experiments and in the 60–70 minute ballooned animals. Variability in the shape of the transmural tissue concentration profiles between animals within a few of the balloon-deendothelialized groups was marked, especially in the 5–15, 5–65, and 60–90 minute ballooned ani-

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**Figure 2.** Transmural profiles of relative tissue concentration of TCA-precipitable radioactivity, averaged for each rabbit, for 5–15, 5–35, and 5–65 minute control experiments, corresponding to 10-, 30-, and 60-minute uptake experiments initiated 5 minutes after sham balloon-deendothelialization, respectively.

**Figure 3.** Transmural profiles of relative tissue concentration of TCA-precipitable radioactivity, averaged for each rabbit, for 5–15, 5–35, and 5–65 minute balloon-deendothelialization experiments, corresponding to 10-, 30-, and 60-minute uptake experiments initiated 5 minutes after deendothelialization.
Figure 4. Transmural profiles of relative tissue concentration of TCA-precipitable radioactivity, averaged for each rabbit, for 60–70 and 60–90 minute balloon-deendothelialization experiments, corresponding to 10- and 30-minute uptake experiments initiated 60 minutes after deendothelialization.

Figure 5. Comparison of grand average profiles for all experiments. Data points were obtained by averaging spatially congruent data points from all animals from each group in Figures 2, 3, and 4. Error bars represent standard errors of the mean.
mals. A peak in concentration within the media occurred in several profiles from the latter two groups. Control animals exhibited relatively consistent transmural concentration profiles in all three groups.

The mean relative tissue concentration ($C_{T}/C_{Po}$) for each aortic sample was calculated by dividing the total amount of TCA-precipitable radioactivity in the media by the volume of the sectioned sample. Mean values for each animal and for each group of animals were similarly calculated. Values of $C_{T}/C_{Po}$ for each of the groups studied are plotted in Figure 6 versus the time after tracer injection. Each data point represents the net relative accumulation of $^{125}$I-albumin. $C_{T}/C_{Po}$ increased monotonically with time for the balloon-deendothelialized animals at a rate much greater than that observed with the control animals. The net accumulation in balloonized animals after comparable time periods of uptake was about 50% greater when $^{125}$I-albumin was injected 60 minutes after balloon-deendothelialization than when tracer was injected 5 minutes after deendothelialization.

A two-way analysis of variance test of the mean relative tissue concentration for individual aortic samples did not detect significant ($\alpha = 0.05$) differences in the values within individual animals of a given group but detected significant ($\alpha = 0.05$) differences in the mean values between animals of a given group relative to random differences in the values within a group of animals. These findings were valid for all groups of animals except the 5–15 minute control experiments. A Duncan’s multiple range test$^{15}$ of $C_{T}/C_{Po}$ for individual aortic samples showed a significant ($\alpha = 0.05$) increase in the values from all balloon groups relative to those of control groups with corresponding tracer circulation times. Duncan’s test also detected a significant ($\alpha = 0.05$) increase in the mean values for the 60–70 and 60–90 minute balloonized animal groups as compared to the 5–15 and 5–35 minute balloonized animal groups, respectively.

**Discussion**

This study represents the first attempt to quantify the uptake of radiolabeled albumin into the deendothelialized aorta in vivo and to provide a quantitative description of its transmural distribution in the injured aortic wall. The balloon catheter was selected for inducing endothelial injury because it removes endothelial cells from the rabbit descending thoracic aorta with minimal damage or distortion to other components of the vessel wall.$^{11,12}$ Transient pressure increase and decrease may occur proximal and distal, respectively, to the inflated balloon during deendothelialization. The controls provide a comparison between the balloon catheter-deendothelialized animals and animals that have not undergone balloon catheter-deendothelialization but are otherwise identically treated. The tissue serial sectioning technique was used in this study to obtain the spatial resolution needed for accurate transmural concentration profiles. Analysis of these concentration profiles across the normal and balloon-deendothelialized rabbit aortic wall provides 1) information on the transport barrier properties of the endothelium, and 2) insights into the possible transport mechanisms for plasma proteins across the aortic wall.

Our results with anesthetized control animals (Figures 2 and 5) are qualitatively consistent with our previous findings with conscious animals.$^9$ Concentration levels were higher and concentration gradients at the luminal border steeper in the 10-minute uptake experiments of our previous study$^9$ as compared to the 5–15 minute control experiments in this study. Furthermore, gradients at the luminal border persisted up to 24 hours after injection,$^9$ whereas the profile was relatively flat in the 5–65 minute control experiments of this study. The absence of steep gradients is consistent with results of another study with anesthetized rabbits infused continuously with isotonic saline for 30 minutes after $^{125}$I-albumin injection.$^{16}$ These findings suggest that subtle differences in transport mechanisms may exist between conscious and anesthetized animals. However, the possibility of experimental artifact in our previous data$^9$...
cannot be ruled out since we obtained very thin (e.g., 2 μm) sections at the luminal surface where plasma contamination is difficult to eliminate.

The mean relative tissue concentrations (\(\bar{C}_T/CPo\)) we measured in the aortic media of control rabbits are compared in Table 1 with data from other studies of labeled 125I-albumin uptake in normal animals. Despite methodological differences, there is reasonable overall agreement between the various studies with rabbits.

The relative tissue concentration of 125I-albumin in the aortic media of ballooned animals (Figures 3–6) increased dramatically to 10 to 40 times that of control animals with corresponding tracer circulation times. These results corroborate the findings of others4–8 that the endothelium represents the primary barrier to macromolecular transport across the arterial wall. Whereas the concentration profiles in the control animals displayed gradients at both the luminal and medial-adventitial borders, these gradients were absent in the ballooned animals. In the 5–15, 5–35 and 60–70 minute ballooned animal groups, the relative tissue concentration was higher near the luminal border than near the medial-adventitial border. The profiles were relatively flat in the 5–65 and 60–90 minute ballooned animals. These results suggest possible changes in the relative magnitudes of the mechanisms for transport of 125I-albumin across the aortic wall upon balloon-deendothelialization. The flatter profiles are consistent with a predominance of convective over diffusive transport across the wall. The variability in shape between profiles and the presence of peaks in some of the profiles may reflect variability between animals and nonuniformities across the arterial wall in the albumin distribution space.

The mean relative tissue concentration, and therefore the net rate of uptake, was about 50% greater in the 10- and 30-minute circulation experiments initiated 60 minutes after deendothelialization than in those initiated 5 minutes after deendothelialization (Figure 6). The surface coverage of the subendothelium by adherent platelets in the descending thoracic aorta increases from less than 5% at 10 seconds after injury to about 80% at 90 minutes following injury.11 If the incomplete platelet monolayer acted as a substantial transport barrier, the 60-70 and 60-90 minute experiments should have provided for a smaller rate of uptake than the 5-15 and 5-35 minute experiments at equivalent tracer circulation times. Since the converse was true, the adherent platelets probably did not serve as a substantial transport barrier to albumin. This interpretation is reasonable in view of a recent theoretical analysis.20
of the diffusion of macromolecules across the arterial wall in the presence of multiple endothelial injuries, which showed that the flux across the wall approaches that for a completely denuded endothelium when there are a large number of small, highly permeable damage sites, and the distance between damage sites is small compared to the thickness of the arterial wall. The same conclusion should also apply when transport is dominated by convection rather than diffusion. In the present study, the gaps between adhering platelets averaged about 1000 nm, which permitted unimpeded passage of 125I-albumin molecules, and the diameter of each platelet was only a small percentage of the aortic wall thickness.

The observation that the rate of uptake of 125I-albumin by the deendothelialized aortic wall increases with time during the 90-minute period following balloon injury suggests that small structural changes may occur in the arterial wall during this time, such as increased edema, which would make the media more permeable to diffusive and/or convective transport mechanisms. Progressive medial edema following balloon injury might lead to an increase in albumin diffusivity within the media and/or in fractional medial volume available to solute, either of which could lead to increased uptake. In a previous study, we observed some edema of the media, but extracellular fluid accumulation was not seen beyond the innermost two or three lamellar units. In vitro studies with carotid arteries suggest that the medidial distribution volume for proteins reflects the properties of the interstitium that may be influenced by externally applied stress, smooth muscle tone, convection through the wall, and the presence of vasoactive agents. An increase with time in edema and albumin distribution space was observed in vitro in the rabbit aorta following intimal damage. 

Our uptake data is in marked contrast to the results of other transport studies carried out after much longer time periods following injury. In these studies, the rate of uptake decreased with time after injury as a result of endothelial regrowth that replaced the transport barrier initially lost upon deendothelialization.

We have carried out a theoretical analysis of 125I-albumin transport in the arterial wall in which a mathematical model was fitted to the 10- and 30-minute 125I-albumin concentration profile data from normal and deendothelialized rabbits to permit quantitative evaluation of transport parameters and assessment of the relative importance of different transport mechanisms. Some of the important qualitative notions obtained from the analysis are summarized as follows: In the normal animal, the endothelium limits the rate of uptake and is probably traversed only by vesicular transport. Within the media, convective and diffusive transport are of comparable importance. When the endothelium is removed, the hydraulic permeability of the arterial wall increases substantially (a finding consistent with experimental observations) to the point where convective transport dominates. In addition, the diffusion coefficient of 125I-albumin does not change significantly after balloon injury. The interplay between diffusive and convective transport in the media largely accounts for the change in shape of the concentration profiles after deendothelialization. However, the possibility that nonhomogeneity in albumin distribution space may influence the change in shape cannot be eliminated. The subendothelium acts only as a partially retentive barrier, as a consequence of which the concentration of albumin and other plasma proteins increases sharply in the intima and medial regions of the arterial wall after deendothelialization. The detailed results of this analysis will be reported in a subsequent publication.

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