The role of mitosis in LDL transport through cultured endothelial cell monolayers

Limary M. Cancel1,2 and John M. Tarbell1

1Department of Biomedical Engineering, The City College of The City University of New York, and 2The Graduate Center of The City University of New York, New York, New York

Submitted 11 May 2010; accepted in final form 14 December 2010

Cancel LM, Tarbell JM. The role of mitosis in LDL transport through cultured endothelial cell monolayers. Am J Physiol Heart Circ Physiol 300: H769–H776, 2011. First published December 17, 2010; doi:10.1152/ajpheart.00445.2010.—We (7) have previously shown that leaky junctions associated with dying or dividing cells are the dominant pathway for LDL transport under convective conditions, accounting for >90% of the transport. We (8) have also recently shown that the permeability of bovine aortic endothelial cell monolayers is highly correlated with their rate of apoptosis and that inhibiting apoptosis lowers the permeability of the monolayers to LDL. To explore the role of mitosis in the leaky junction pathway, the microtubule-stabilizing agent paclitaxel was used to alter the rate of mitosis, and LDL flux and water flux (J_W) were measured. Control monolayers had an average mitosis rate of 0.029%. Treatment with paclitaxel (2.5 μM) for 1.5, 3, 4.5, or 6 h yielded increasing rates of mitosis ranging from 0.099% to 1.03%. The convective permeability of LDL (P_c) increased up to fivefold, whereas J_W increased up to threefold, over this range of mitosis rates. We found strong correlations between the mitosis rate and both P_c and J_W. However, compared with our previous apoptosis study (8), we found that mitosis was only half as effective as apoptosis in increasing P_c. The results led us to conclude that while mitosis-related leaky junctions might play a role in the initial infiltration of LDL into the artery wall, the progression of atherosclerosis might be more closely correlated with apoptosis-related leaky junctions.

low-density lipoprotein permeability; bovine aortic endothelial cells; water flux; leaky junctions; mitosis

THE FIRST EVENT in the cascade of processes leading to atherosclerotic lesion formation is lipid infiltration and accumulation within the arterial wall (41). A relationship between enhanced endothelial permeability to LDL and the localization of atherosclerotic plaques is well established (16, 33). There are three potential pathways for the transport of macromolecules across the endothelium: transcytosis in vesicles, paracellular transport through breaks in the tight junction strand, and the leaky junction pathway associated with cells undergoing mitosis or apoptosis. Previous in vivo (13, 28, 29) and theoretical (48) studies have supported the importance of the leaky junction pathway in LDL and albumin transport across the endothelium. In studies by Lin et al. (30) and Chien et al. (13), aortic sections were stained with hematoxylin to identify cells in the M phase of the cell cycle using morphological criteria. Roughly 1 in 3,000 cells was found to be mitotic, but nearly all of those had an Evans blue-albumin (EBA) or Lucifer yellow (LY)-LDL leakage spot associated with it (99% for EBA and 80% for LY-LDL). Mitotic cells accounted for 30% of all EBA leaks and 45% of all LY-LDL leaks.

Observations that hemodynamic factors usually associated with the development of atherosclerosis also affect endothelial cell (EC) proliferation provide indirect evidence for its importance in atherogenesis. Atherosclerotic plaques tend to be localized in regions of low shear stress and disturbed flow (17, 25, 32). Several studies (19, 26) have demonstrated that high levels of steady shear stress reduce the rate of EC proliferation. Chien (12) measured EC proliferation using bromodeoxyuridine (BrdU) incorporation in a step flow chamber. After 24 h of flow at 12 dyn/cm², BrdU incorporation was enhanced in the region of disturbed flow, whereas it was much lower in the downstream laminar flow region.

A recent in vitro study by our laboratory (7) demonstrated that leaky junctions are the dominant transport pathway for LDL under convective conditions, when transport is driven by transendothelial water flux induced by the pressure differential across the endothelial layer. In that study, we used a model consisting of bovine aortic ECs (BAECs) plated onto porous polyester filters and measured the flux of albumin, water (J_W), and LDL across the endothelial monolayers under convective conditions. Leaky junctions accounted for 90.9% of LDL transport, with the remainder going through vesicles. These results are consistent with in vivo observations showing that vesicles do not contribute significantly to LDL uptake in arteries (37, 49). We (8) have also recently shown that the permeability of BAEC monolayers is highly correlated with their rate of apoptosis and that inhibiting apoptosis lowers the permeability of monolayers to LDL. In the present study, we used our in vitro model to investigate the role of mitosis in LDL transport under convective conditions. BAEC monolayers were treated with the microtubule-stabilizing drug paclitaxel, a taxane used in the treatment of lung, ovarian, and breast cancer (38) and in the prevention of restenosis of coronary stents (14, 42), for different periods of time to obtain elevated rates of mitosis. Paclitaxel binds microtubules (39), thus hyperstabilizing the microtubule network and arresting cells in the M phase (23). LDL flux was measured using an automated fluorometer system, and J_W was measured using a bubble tracker apparatus. We found correlations between the mitosis rate and convective and diffusive LDL permeabilities (P_c and P_o, respectively) as well as J_W across the monolayers. Our results indicate a linear relationship between the mitosis rate and each of these three transport properties.

MATERIALS AND METHODS

Materials. The following chemicals were obtained from Sigma (St. Louis, MO): minimum essential medium (MEM), fibronectin, BSA, L-glutamine, penicillin-streptomycin, and paclitaxel. Phenol-red free
Experiments were carried out on monolayers 4 – 6 days postplating.

**Cell culture.** BAECs were purchased from VEC Technologies (Rensselaer, NY) and grown in MEM supplemented with 10% FBS, 1% t-glutamine, and 1% penicillin-streptomycin. For transport experiments, cells were plated onto fibronectin-coated Transwell membranes (Corning, Acton, MA) at a density of 1.25 × 10^5 cells/cm². Experiments were carried out on monolayers 4–6 days postplating.

**Cell treatment and measurement of mitosis rates.** An increased rate of mitosis was obtained by incubating BAEC monolayers with the microtubule stabilizer paclitaxel at a concentration of 2.5 μM for 1.5, 3, 4.5, or 6 h (45). Mitosis rates were determined using anti-phosphoSer/Thr-Pro (MPM-2; Upstate Biotechnology, Lake Placid, NY), a primary antibody that binds to a phospho-amino acid-containing epitope present on >50 proteins of eukaryotic cells in the M phase. Briefly, cells were washed with PBS, fixed with 4% paraformaldehyde for 10 min, permeabilized with ice-cold methanol for 6 min, and blocked with 1% BSA in PBS. After being washed two times with PBS, monolayers were incubated with primary antibody (MPM-2; 1:200 in blocking solution) for 2 h at room temperature or overnight at 4°C. Monolayers were then washed three times with PBS and incubated with the secondary antibody (Alexa fluor594 goat anti-rabbit IgG, Invitro- gen, Carlsbad, CA) for 1 h at room temperature. Monolayers were then washed five times with PBS and incubated with the primary antibody (MPM-2, 1:200 in blocking solution) for 2 h at room temperature or overnight at 4°C. Monolayers were then washed two times with PBS and incubated with the secondary antibody (Alexa fluor488 rabbit anti- mouse, 1:200 in PBS) for 1.5 h at room temperature. Monolayers were washed three times in PBS before being imaged. The mitosis rate was defined as the number of MPM-2-positive cells divided by the total number of cells on the filter.

To check that paclitaxel treatment did not induce apoptosis, the In Situ Cell Death Detection kit (TMR red) from Roche (Indianapolis, IN) was used to measure the apoptosis rate on some monolayers. This assay uses the TUNEL technique to identify apoptotic cells. Cells were fixed with 4% paraformaldehyde for 1 h, permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min, and then incubated with the TUNEL reaction mixture for 1 h at 37°C. Cells were then washed three times with PBS before being imaged.

**Immunostaining of junction proteins.** BAEC monolayers were grown in the same manner as for transport experiments. Monolayers were washed twice with PBS, fixed in 1% paraformaldehyde for 10 min, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and blocked with 10% BSA and 0.1% Triton X-100 in PBS for 1 h. After being washed with PBS, monolayers were incubated with the primary antibody for vascular endothelial (VE)-cadherin (3:200, Cell Signaling, Beverly, MA) for 3 h at room temperature or overnight at 4°C. Monolayers were then washed five times with PBS and incubated with the secondary antibody (Alexa fluor594 goat anti-rabbit IgG, Invitrogen, Carlsbad, CA) for 1 h at room temperature. Monolayers were washed four times in PBS and refixed with 4% paraformaldehyde for 10 min. The MPM-2 assay described above was then performed to co-stain for mitotic cells.

**Measurement of solute flux and Jv.** Dil-LDL flux was measured using an automated fluorometer system previously developed in our laboratory (2) and extensively described (7, 8). Briefly, a Transwell filter containing the BAEC monolayer was sealed within a transport chamber to form a luminal (top) and abluminal (bottom) compartment. An aluminum support ring and an O-ring helped create a tight seal within the chamber. The abluminal compartment was connected to a fluid reservoir via tygon tubing and borosilicate glass. The reservoir could be lowered to apply a hydrostatic pressure differential across the monolayer. Both the luminal compartment and abluminal reservoir were continuously supplied with 5% CO2-95% air to maintain the medium at physiological pH. The experimental apparatus, excluding the laser source and the emission detector, were housed inside a Plexiglas box. A hairdryer attached to a temperature controller maintained the air temperature inside the box at 37°C.

Each filter was rinsed twice with experimental medium (1% BSA in phenol red-free MEM) before being inserted into the chambers. A solution of 5 μg/ml Dil-LDL was added to the luminal compartment. Each transport experiment consisted of a 1-h equilibration period (no pressure differential), the abluminal reservoir was then lowered to apply a 10-cmH2O pressure differential (to drive a physiological amount of Jv across the monolayer), and data were collected for 1 h. Finally, the abluminal reservoir was raised to its original level, and data were collected for 1 h under diffusive conditions (no pressure differential). Permeability was calculated as follows:

\[
P_{cv} = \frac{(\Delta C_v/\Delta t) \times V_a}{C_i \times A}
\]

where \(\Delta C_v/\Delta t\) is the change in abluminal concentration with respect to time, \(V_a\) is the fluid volume in the abluminal compartment, \(C_i\) is the concentration in the luminal compartment, and \(A\) is the area of the filter.

\(J_v\) was measured simultaneously with solute flux. When a 10-cmH2O differential pressure was applied, \(J_v\) was measured by tracking the position of an air bubble that was inserted into the glass tubing. The bubble was monitored using a spectrophotometer, which followed the air-liquid interface in the glass tube.

The bubble displacement data were used to compute \(J_v\) values using the following equation:

\[
J_v/A = \frac{\Delta d/\Delta t \times F}{A}
\]

where \(\Delta d/\Delta t\) is the bubble displacement per unit time and \(F\) is a tube calibration factor (fluid volume per unit length of tubing).

**Statistical analysis.** Minitab (State College, PA) was used to calculate linear regressions between mitosis and permeability values and to perform a power analysis for the gap data shown in Table 1.

### Table 1. Association of leakage spots or increased \(P_v\) with mitotic cells and tricellular corners

<table>
<thead>
<tr>
<th>Description</th>
<th>Mitotic Cells</th>
<th>Percentage of Total Sites With Leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency, %</td>
<td>Percent with leakage</td>
</tr>
<tr>
<td></td>
<td>Mitotic</td>
<td>Tricellular</td>
</tr>
<tr>
<td>Present study</td>
<td>Bovine aortic endothelial cells; estimated leakage spots</td>
<td>0.143 ± 0.03</td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paclitaxel (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truskey et al.</td>
<td>Rabbit aorta; increased (P_v)</td>
<td>0.011</td>
</tr>
<tr>
<td>Lin et al.</td>
<td>Rat aorta; leakage spots</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Leakage spots for the present study were estimated using observations of gaps in cell-cell junctions. *The power for a test of the means, with \(\alpha = 0.05\), was 0.84.* \(\alpha\)Truskey et al. and Lin et al. did not look at tricellular corners; therefore, the unexplained leakage spots were assigned to the “other” category. \(P_v\), convective permeability.
Correlations were evaluated using the Pearson product-moment correlation coefficient, which was also calculated using Minitab. A comparison between the correlations was made using Fisher’s method.

Each correlation was normalized by the average of the controls for each experimental set using the following formula:

\[
\left(\frac{y}{y_c}\right) = \frac{a x_c}{y_c} + \frac{b}{y_c}
\]

where \(y_c\) is the average \(P_e, P_o,\) or \(J_v\) for control monolayers in each experimental set; \(x_c\) is the average mitosis rate for control monolayers in each experimental set; \((y/y_c)\) is the normalized \(P_e, P_o,\) or \(J_v;\) \((ax/y_c)\) is the slope of the normalized equation; \((b/y_c)\) is the intercept of the normalized equation; and \(a\) and \(b\) are the slope and intercept of the original equation, respectively.

RESULTS

Effect of paclitaxel on mitosis rate and LDL permeability. \(P_e\) and \(P_o\) values of control and paclitaxel-treated monolayers were measured using our transport system. After each transport experiment, monolayers were fixed with 4% paraformaldehyde, and the mitosis assay was performed. The mitosis rate of each BAEC monolayer was plotted against its corresponding permeability (Figs. 1 and 2).

The mitosis rate of BAEC monolayers maintained in growth media (10% FBS-MEM) ranged between 0.008% and 0.093% on day 5 after plating. To increase mitosis rates, BAEC monolayers were incubated with paclitaxel at a concentration of 2.5 \(\mu\)M for 1.5, 3, 4.5, or 6 h. Paclitaxel does not induce additional mitosis but instead arrests the cells in the M phase by hyperstabilizing the structure of microtubules. Mitosis in ECs is expected to last \(\sim\)1 h (13). Therefore, we expected an about onefold increase in mitosis per hour of incubation. However, the increase in mitosis greatly depended both on the basal rate of mitosis at the time of incubation and on the duration of the incubation period. For this reason, results from each incubation period were not pooled together and averaged but instead plotted individually against the permeability measured for each particular monolayer. When treated with paclitaxel at the indicated times, the mitosis rate ranged between 0.099% and 1.03% (Figs. 1 and 2).

Paclitaxel has also been shown to induce apoptosis in certain cell types, including ECs, depending on the concentration of the drug.

Fig. 1. Correlation between mitosis rate and diffusive permeability (\(P_o\)). The plot shows the mitosis rate and \(P_o\) for control monolayers and monolayers treated with paclitaxel for 1.5, 3, 4.5, or 6 h. The inset shows the correlation when only control monolayers are considered. The Pearson product-moment correlation coefficient for the full range was 0.696 (\(P < 0.0005\)). For the data in the inset, the correlation was not significant.

Fig. 2. Correlation between mitosis rate and convective permeability (\(P_e\)). The plot shows the mitosis rate and \(P_e\) for control monolayers and monolayers treated with paclitaxel for 1.5, 3, 4.5, or 6 h. The inset shows the correlation when only control monolayers are considered. The Pearson product-moment correlation coefficient for the full range was 0.774 (\(P < 0.0005\)). For the data in the inset, the Pearson product-moment correlation coefficient was 0.499 (\(P < 0.021\)).
used and the exposure time (35, 47). To check that our treatment did not induce apoptosis in BAECs, we used the TUNEL technique to measure apoptosis in representative control and paclitaxel-treated monolayers. The apoptosis rate was $0.47 \pm 0.03\%$ for control monolayers, similar to our previous results (8) and to rates of cell death in the normal rat aorta (30). Apoptosis rates were $0.45 \pm 0.03\%$ and $0.50 \pm 0.06\%$ for monolayers treated with paclitaxel for 4.5 and 6 h, respectively. There were no significant differences in the apoptosis rate between these control and treated monolayers ($P > 0.6$). In the results that follow, it should be understood that the permeabilities for all monolayers (control and paclitaxel treated) are the result of this baseline apoptosis rate and varying mitosis rates.

The average $P_o$ of control monolayers was $2.8 \times 10^{-7} \pm 2.5 \times 10^{-8} \text{ cm/s}$, similar to previous measurements (7, 8). Increasing the mitosis rate had a small but significant effect on $P_o$ to LDL, as shown in the correlation in Fig. 1. The Pearson correlation coefficient was calculated to be 0.696 with a $P$ value of $<0.0005$. The inset in Fig. 1 shows the correlation between mitosis and $P_o$ for the control monolayers alone. The Pearson correlation coefficient for controls alone was not significant. The average $P_e$ of control monolayers was $1.71 \times 10^{-6} \pm 1.50 \times 10^{-7} \text{ cm/s}$, consistent with previous measurements (7, 8). $P_e$ was significantly increased by increasing mitosis rates with paclitaxel, as shown in Fig. 2. The Pearson correlation coefficient was 0.774 with a $P$ value of $<0.0005$. The inset in Fig. 2 shows the correlation between mitosis and $P_e$ for control monolayers alone. The Pearson correlation coefficient for controls alone was 0.499 with a $P$ value of 0.021. A statistical comparison of the two full correlations (convective vs. diffusive) revealed that whereas there were no significant differences between the correlation coefficients (i.e., we could predict both values equally well), the slopes of the correlations were significantly different ($P < 0.0001$).

Effect of paclitaxel on $J_v$. $J_v$ values across control and paclitaxel-treated monolayers were measured simultaneously with permeability, as described in MATERIALS AND METHODS. Figure 3 shows the effect of paclitaxel on $J_v$. Control monolayers had an average $J_v$ of $3.24 \times 10^{-6} \pm 2.39 \times 10^{-7} \text{ cm/s}$. Increasing the mitosis rate significantly increased $J_v$, as shown in Fig. 3. The Pearson correlation coefficient was 0.795 with a $P$ value of $<0.0005$. The inset in Fig. 3 shows the correlation between mitosis and $J_v$ for control monolayers alone. The Pearson correlation coefficient for controls alone was 0.482 with a $P$ value of 0.023.

Colocalization of endothelial gap formation with mitosis. BAEC monolayers were immunostained for the adherens junction protein VE-cadherin and costained for mitosis to assess the colocalization of gaps in the cell-cell junction with mitosis. Representative photomicrographs are shown in Fig. 4. Mitotic cells in control monolayers appeared, in most cases, to be spread out and flattened, whereas paclitaxel-treated mitotic cells appeared rounded. This rounded morphology is characteristic of cells during metaphase (5). Consistent with these results, paclitaxel-treated cells have been shown to arrest in metaphase (23) because the spindle-assembly checkpoint ensures that cells do not enter anaphase until all chromosomes are attached to both poles of the spindle, a process highly dependent on microtubule dynamics (1).

The total number of mitotic cells and gaps in 7 fields/monolayer (0.044 mm$^2$/field) was quantified for four control and four paclitaxel-treated monolayers. All fields analyzed contained at least one mitotic cell. A gap was defined as a clear opening in the cell-cell junction, normally appearing as a dark region between cells (arrows in Fig. 4). In addition to quantifying gaps associated with mitotic cells, we also identified tricellular corners, which occur where three cells meet (arrowhead in Fig. 4A), as well as other gaps. Tight junctions have been shown to be discontinuous at tricellular corners (6, 46), and transendothelial migration of neutrophils was found to occur preferentially at the tricellular corner (6), suggesting that tricellular corners might also constitute a leaky junction. However, it is not known whether the cleft between these discontinuities contains a glycocalyx such as is present in the breaks in the tight junction strand.

Since all camera fields (360 cells/field) analyzed contained at least one mitotic cell, the rate obtained from adding up these fields was artificially high. Therefore, the mitosis rate for each monolayer was obtained from a count of all mitotic cells in the entire filter. Most mitotic cells were found to colocalize with an endothelial gap (87 ± 5% and 93 ± 7% for control and paclitaxel-treated monolayers, respectively). However, most gaps did not colocalize with a mitotic cell. Mitotic cells accounted for 28.7 ± 0.7% and 21.1 ± 5.8% of all the gaps, whereas tricellular corners accounted for 19.6 ± 3.8% and 16.7 ±
2.1% of all the gaps in control and paclitaxel-treated monolayers, respectively.

To compare our gap counts to leakage spot colocalization studies in the literature, we estimated the number of distinct spots our gaps would produce. In vivo, solute leakage spots are formed due to, among other factors, lateral convection within the endothelial basement membrane arising from the difference in permeability between the basement membrane and the internal elastic lamina (IEL). In our in vitro model there is no IEL, and the Transwell filter in which the cells are grown is porous enough to allow excellent transport of the solutes into the bottom compartment of our transport chamber. This is necessary for the accurate measurement of transport properties; however, it prevents the formation of the leakage spots observed in vivo. Lin et al. (30) reported LY-LDL leakage spots around mitotic cells to be slightly larger than the size of one cell. Truskey et al. (44) observed focal regions of increased 125I-labeled LDL permeability, some around mitotic cells, to be two or three cells in diameter. Taking these observations as a guide, we estimated that each gap would produce a spot 1.5 times the surface area of a cell and counted the number of distinct spots our gaps would produce. Where a mitotic gap and another type of gap would both produce a spot covering the same area, the spot was assigned to the mitotic cell. The results are shown in Table 1. In our control monolayers, 82 ± 8% of mitotic cells were associated with an estimated leakage spot accounting for 34 ± 2% of all leaks. In paclitaxel-treated monolayers, 83 ± 4% of mitotic cells were associated with leaks, accounting for 31 ± 3% of all leaks.

**DISCUSSION**

The aim of this study was to show that, as part of the leaky junction pathway, mitosis rates dictate, in part, the permeability of EC monolayers to LDL under convective conditions. To this end, we used paclitaxel to induce elevated rates of mitosis. Verin et al. (45) exposed bovine pulmonary artery ECs to paclitaxel at 2.5 μM for up to 4 h and found that it had no effect on the transendothelial electrical resistance (TER) of cell monolayers. In the same study, the microtubule-disrupting agents nocodazole and vinblastine were shown to increase TER and stress fiber formation, resulting in cell contraction and the formation of gaps between cells. These effects were attenuated by the addition of paclitaxel (45). These results suggest that paclitaxel does not affect the junctional complex between ECs. In the present study, however, we found an effect of paclitaxel on both permeability and $J_v$ associated with changes in the cell-cell junction around mitotic cells. The apparent disparity between our results and those in Verin et al. (45) can be explained by noting that only the junctions around mitotic cells are being affected. Even at the highest mitosis rate obtained with paclitaxel treatment in the present study, only 1 in every 100 cells is arrested in the M phase and therefore has an altered junction. These altered junctions constitute a significant increase in the area available for the transport for large molecules (such as LDL) that cannot easily cross the endothelial layer through any other pathway. They also provide a pathway for $J_v$ with a much lower hydraulic resistance than the normal junction. Ions, the basis for TER measurement, have the entire length of the cell-cell junction (including the tight junction) available for transport. Therefore, it is not expected that 1 altered junction for every 100 cells would significantly increase their transport.

As in our apoptosis study (8), we sought to observe the colocalization of endothelial gap formation with mitotic cells. BAEC monolayers were costained with the adherens junction protein VE-cadherin to identity gaps and MPM-2 for mitotic cells (Fig. 4). Despite the small number of cells analyzed (~2,000 cells each for control and paclitaxel-treated monolay-
shown to degrade the EC glycocalyx, leading to increased secreted primarily by macrophages and monocytes, has been cells (e.g., macrophages). TNF-α, a proinflammatory cytokine, a result of blood flow, that have been shown to affect endo-
thelial function (27, 34). Cells in vivo are also exposed to the present study. In vivo, ECs are exposed to mechanical forces as the relationships between mitosis and permeability observed in the present study. In vitro, the complex environment of cells in vivo may alter the results were similar to previous in vivo studies of the association of LDL leakage with mitosis (Table 1). Truskey et al. (44) studied the associations of mitotic cells with increased permeability of 125I-labeled LDL in the rabbit aorta. They found that 65% of mitotic cells had increased permeability, accounting for 25% of all permeable areas. Lin et al. (30) studied the association of LY-LDL with mitosis in the rat aorta. In that study, 80.5% of mitotic cells were found to colocalize with LY-LDL leakage spots, accounting for 45.3% of all leakage spots. In the present study, we found no significant differences in the colocalization of estimated leakage spots between control and paclitaxel-treated monolayers. Similarly, there were no significant differences in the percentage of total leaks associated with mitosis between the two groups. One previous study (22) examined the frequency of mitotic cells around branching points in the normal rabbit aorta and their association with increased 125I-labeled LDL permeability. They found that while the frequency of mitotic cells away from intercostal branches (0.09 ± 0.04 mitotic cells/mm²) was substantially lower than that around intercostal branches (0.49 ± 0.23 mitotic cells/mm²), the difference was not statistically significant. They also found that 78% of mitotic cells showed increased permeability, accounting for 13% of all sites with increased permeability. Mitotic cells accounted for the same percentage of permeable sites both at and away from branch sites.

The main limitation of this study is that it was performed in vitro. The complex environment of cells in vivo may alter the relationships between mitosis and permeability observed in the present study. In vivo, ECs are exposed to mechanical forces as a result of blood flow, that have been shown to affect endothelial function (27, 34). Cells in vivo are also exposed to the effects of inflammatory mediators (e.g., TNF-α) and of other cells (e.g., macrophages). TNF-α, a proinflammatory cytokine secreted primarily by macrophages and monocytes, has been shown to degrade the EC glycocalyx, leading to increased Jv, and the penetration of macromolecules into the glycocalyx layer (11, 21). While a previous study in our laboratory (31) demonstrated the existence of a functional glycocalyx in our BAEC model, the effects of its degradation on the transport of LDL and on the mitosis correlations established here were not assessed in the present study.

The main results from the present study are the correlations obtained for the association of mitosis with Pe, Po, and Jv. In Fig. 5, these correlations were normalized to allow a direct comparison between all three transport parameters. As expected, the main effect of mitosis was in the Pe, whereas there was only a small, but significant, effect on Po. A statistical comparison of the two permeability correlations revealed that the effect of mitosis on permeability was significantly stronger for Pe than for Po. This plot also shows that to increase each transport property by 2-fold, the mitosis rate would have to be increased by 10-, 18-, and 77-fold for Pe, Jv, and Po, respectively.

Few studies have measured mitosis rates in atheroprone areas of the vasculature. Wright (50) measured the incorporation of [3H]thymidine over the length of the aorta of guinea pigs. The mitotic index varied between 0.54% and 1.23%, with the highest mitotic index found to be in the aortic arch. Around the mouths of branches, [3H]thymidine uptake was significantly higher, by 1.7-fold, than in control areas at the same level but remote from the branch. When a silver clip was used to create an arterial constriction, a 3.7-fold increase in thymidine uptake was found in the stenosed area. Caplan and Schwartz (9) found a 1.4-fold increase in the [3H]thymidine labeling index in areas of EBA uptake in the pig aorta. Schwartz (40) measured [3H]thymidine uptake in the rat aorta and found clusters of replicating cells. While not all of these clusters were correlated with areas of disturbed flow, the clusters had an average mitotic index that was twice that of the entire population average. Since [3H]thymidine incorporates into the nuclei of cells in the DNA S phase, these rates cannot be directly compared with our M phase rates. In addition, cells in the S phase are not likely to yet be leaky. However, these results do show that atheroprone areas exhibit higher cell turnover rates than protected areas such as the straight part of the aorta.

Better studies are needed to determine the fraction of cells in the M phase (when the junctions are likely to be leaky) in atheroprone areas of the aorta as well as during early lesion formation. However, the results summarized above seem to indicate that the increase in mitosis rates in these areas would likely be lower than 10-fold. If we consider a range of increase in mitosis between two- and fivefold above control, our results

![Fig. 5. Effect of mitosis rate on transport properties. The data shown in Figs. 1–3 were normalized by the average control values for each set. The inset shows detail at the low range of (normalized) mitosis.](image-url)
indicate increases in permeability between 15% and 47% (inset in Fig. 5). These results are in good agreement with the previous in vivo study of Truskey et al. (44), in which they found that permeable areas associated with mitotic cells had LDL permeability 26–47% higher than regions not associated with mitotic cells. In experiments with 131I-labeled albumin and 131I-labeled fibrinogen, the blue areas associated with increased cell turnover had permeabilities 66% and 64% higher, respectively, than white areas (3, 4).

In conclusion, we found a strong linear relationship between the mitosis rate of EC monolayers and their \( P_e \) to LDL. The predicted increases in LDL permeability are in agreement with values observed in vivo in areas of increased cell turnover. Comparing the correlation between mitosis and \( P_e \) obtained here with that obtained between apoptosis and \( P_e \) in our previous study (8) reveals that mitosis is half as effective as apoptosis in increasing \( P_e \) (Table 2). In addition, taking into account the physiological (and pathological) ranges of mitosis and apoptosis rates, we found that apoptosis increases much more than mitosis in diseased areas. Increased mitosis rates are found in atheroprotean areas before any lesion has formed and are about two to four times the baseline rate, as summarized above. Apoptosis rates in these areas are also about fourfold greater than in protected areas (18) before any lesion has formed. Once the lesion has formed, the highest apoptosis rates are found around the plaque stenosis (43) and can reach up to 50 times the baseline. Several studies of cell proliferation in plaques have reported increased mitosis of smooth muscle cells as well as other cells of undetermined type in the intima but not of the luminal ECs covering the plaque (10). In addition, most mediators of the inflammatory process associated with plaque formation (e.g., TNF-\( \alpha \) and oxLDL) have been shown to increase EC apoptosis (15, 20, 24, 36). Taken together, these results indicate that EC mitosis may play an important role in the initial infiltration of LDL into the artery wall and therefore the initiation of the inflammatory response. However, the progression of atherosclerosis might be more closely correlated with EC apoptosis.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-57093.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


