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# Local and systemic drug competition in drug-eluting stent tissue deposition properties

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**Abstract**

The efficacy of drug-eluting stents (DES) requires delivery of potent compounds directly to the underlying arterial tissue. The commercially available DES drugs rapamycin and paclitaxel bind specifically to their respective therapeutic targets, FKBP12 and polymerized microtubules, while also associating in a more general manner with other tissue elements. As it is binding that provides biological effect the question arises as to whether other locally released or systemically circulating drugs can displace DES drugs from their tissue binding domains. Specific and general binding sites for both drugs are distributed across the media and adventitia with higher specific binding associated with the higher specific binding site densities in the media.

The ability of rapamycin and paclitaxel to compete for specific protein binding and general tissue deposition was assessed for both compounds simultaneously and in the presence of other commonly administered cardiac drugs. Drugs classically used to treat standard cardiovascular diseases, such as hypertension and hypercoaguability, displace rapamycin and paclitaxel from general binding sites, possibly decreasing tissue reserve capacity for locally delivered drugs. Paclitaxel and rapamycin do not affect the other's binding to their biologically relevant specific protein targets, but can generally displace each other from tissue at three log order molar excess, decreasing arterials loads by greater than 50%. Local competitive binding therefore should not limit the placement of rapamycin and paclitaxel eluting stents in close proximity.
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*Keywords:* Stents; Drug delivery; Rapamycin; Paclitaxel

## 1. Introduction

The viability of candidate drug-eluting stents has been linked to the properties of the drugs they release. Successful devices elute compounds that penetrate and can be retained in tissue at high local concen-

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trations. Failed devices seem to have considered drugs that are rapidly cleared from arterial tissue [1–4]. A hydrophilic compound like heparin cannot be made to stay in tissue to exert its numerous vasoactive effects over clinically viable time periods [5–7]. Rapamycin and paclitaxel have emerged as the two leading clinical therapies for stent delivery in major part because aside from their putative biological effects their physiochemical properties favor prolonged tissue retention. Paclitaxel binds specifically to a heterodimer of tubulin and in a more general manner to a range of plasma and tissue bound proteins. Rapamycin exhibits a similar effect as it specifically associates with the FK506 binding protein complex (FKBP) and binds generally to a wide range of nonspecific proteins. Neither rapamycin or paclitaxel are large compounds. They are both less than 1000 Da. Yet, their insolubility and the immediate impact of protein binding markedly reduces convection velocities compared to much larger, but much more readily diffusible and soluble compounds, like heparin. The confluence of these physical forces creates a relatively high overall partitioning within arterial tissues. Though more similar to each other than to other hydrophilic drugs the long term retention, elution from and distribution within arteries do differ significantly for these two compounds. It has been hypothesized that this difference derives in part from the dissimilar distribution in FKBP [8,9] and polymerized microtubules [10].

As local binding is critical to local effect two questions arise with the increasing use of these devices. First, as the number of stents implanted per procedure and in a given artery rises concern has been raised as to the potential interactions of the same or different drugs eluted from multiple adjacent or overlapping DES. Second, will the powerful circulating medications patients receive potentially change the binding of drugs eluted from the stents. In part these two questions pose the polar ends of a spectrum of issues. In the first case the question is whether high local tissue levels of drugs that have almost undetectable circulating concentration compete with each other when both are directed to the same defined target tissue, and the second is whether high steady state circulating levels of a systemically administered drug can compete with the tissue binding of a locally eluted compound. Accordingly, we examined the general and specific tissue binding of rapamycin and paclitaxel in the presence of added amounts of these compounds or common systemically administered cardiac drugs.

## 2. Methods

### 2.1. Tissue binding competition assays

Rapamycin was generously donated by Johnson and Johnson/Cordis, radiolabeled Paclitaxel was provided by Vitrax and unlabeled Paclitaxel was from LC Laboratories. For all tissue experiments radiolabeled drugs were loaded at $10^{-6}$ M in 1 cm$^2$ fresh bovine calf carotid artery tissue. Rapamycin blocked general binding sites for paclitaxel tissue uptake and vice versa. Specific binding was inhibited with FK506 (Eton Bioscience) and colchicine (Sigma). FK506 displaces rapamycin as it binds to the same site on the FKBP protein, and colchicine displaces paclitaxel binding by stabilizing depolymerized microtubules. All samples were processed and assayed using previously described standard liquid scintillation techniques [1]. Statistical significance was evaluated using a two-tailed Student's *t*-test with comparison to control cases.

Drug and binding site distribution radially through the arterial wall were correlated. Radiolabeled drug content in 40 μm transmural sections was spatially mapped to tissue immunostained for the binding proteins- tubulin or FKBP (both antibodies from BD Bioscience, San Jose, CA). FK506, colchicine or the indicated non-labeled control competitor drugs (labeled rapamycin plus non-labeled paclitaxel and vice versa) were loaded in tissue at a three log order molar excess over the labeled drug concentration to maximize competitive displacement effects.

### 2.2. Specific protein binding assays

Combinations of radiolabled rapamycin and human wild type FKBP protein (generously donated by Ariad, Cambridge, MA) at 1 : 1 molar ratios were equilibrated for one hour with competitor drugs at molar ratios ranging from 1 : 1000 to 1000 : 1. Solutions were purified through a lipophilic Sephadex column (Sigma-Aldrich, USA) to isolate labeled

rapamycin bound to FKBP. Paclitaxel polymerization assays were performed using standard optical density spectrophotometry methods with measurements at 340 nm and tubulin concentrations of 1 mg/ml. In brief, purified tubulin (Cytoskeleton, Denver, CO) was mixed with GTP, paclitaxel and a competitor drug (with the exception of the control sample). Samples were allowed to polymerize for one hour at 32 °C with optical density measurements made at the beginning and end of the experiment. All data were corrected for absorbance of the competitor drugs by subtracting off the signal contributions of the individual drugs.

## 3. Results

### *3.1. Drug competition in local delivery*

Bovine carotid arterial tissue preparations were incubated for 24 h in $10^{-6}$ M paclitaxel or rapamycin along with the indicated competitor drug at a three log order molar excess (Fig. 1A). Rapamycin displaces 35% of the labeled paclitaxel from tissue specimens while paclitaxel blocks only 50% of rapamycin binding. FK506 has no significant effect on paclitaxel loading but eliminates 50% of the binding capacity for rapamycin, likely through occupying sites on the specific binding protein FKBP in the tissue. Similarly colchicine significantly decreases paclitaxel uptake by ~50% but had a statistically insignificant effect on rapamycin uptake.

To elucidate the mechanisms of displacement tissue samples were preloaded with only the competitor drugs for 24 h. Following this incubation period, tissue was moved to a separate bath containing $10^{-6}$ M paclitaxel or rapamycin for an additional 24 h. Only colchicine pre-incubation reduced paclitaxel binding, otherwise the tissue loading did not significantly differ from no-competition controls (Fig. 1B). Previously we have shown that hydrophobic species such as paclitaxel and rapamycin, and hydrophilic dextrans [1] elute out of tissue to steady state levels over a period of approximately 12 h. These results imply that the pre-incubated competitor drugs elute out of tissue and/or are displaced by paclitaxel and rapamycin, allowing the labeled drugs to achieve their full tissue loading potential. In the colchicine–paclitaxel case, it appears that colchicine remains

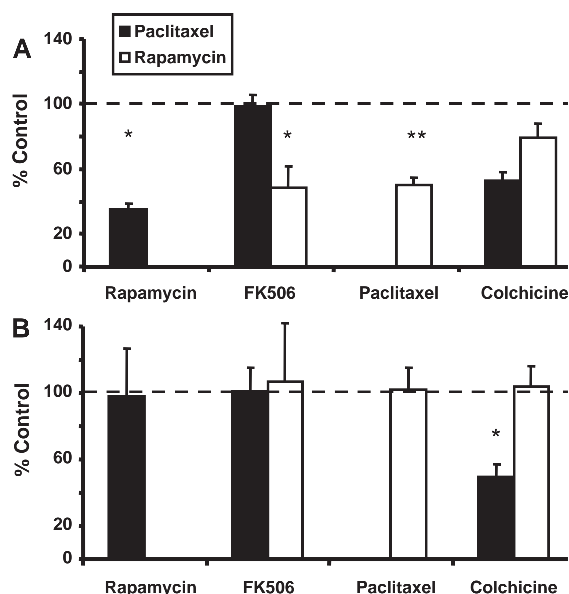


Fig. 1. (A) Percentage of paclitaxel (■) and rapamycin (□) in tissue relative to no-competition controls after 24 h when simultaneously loaded with various competitor drugs (indicated in the figure) and (B) when tissue was exposed to competitor drug for 24 h followed by a separate 24-h loading of the labeled drugs. (*) indicates a statistically significant difference ($p<0.05$ with a two-tailed Student's $t$-test) from the no-competition control. Standard deviations are plotted with the data set.

sufficiently associated with tissue proteins to reduce the overall paclitaxel tissue levels.

Dose response curves were constructed to evaluate local competition between paclitaxel and rapamycin. Paclitaxel (Fig. 2A) and rapamycin (Fig. 2B) at $10^{-6}$ M were both simultaneously incubated with varying doses of paclitaxel and rapamycin. When labeled paclitaxel is loaded with unlabeled paclitaxel, significant displacement of the labeled drug is noted at concentrations between $10^{-6}$ and $10^{-5}$ M. For paclitaxel loading with rapamycin, significant displacement of drug does not occur until the concentration of rapamycin is between $10^{-4}$ and $10^{-3}$ M. Similar results are observed when assaying rapamycin characteristics. For both cases, three log order molar excess of unmatched drug is required to reduce binding of the labeled drug significantly. Arterial concentrations from stent delivery in porcine models typically yield tissue concentrations between $10^{-8}$ and $10^{-7}$ M. At $10^{-8}$ M loading of labeled drug, competition drug concentrations still must reach

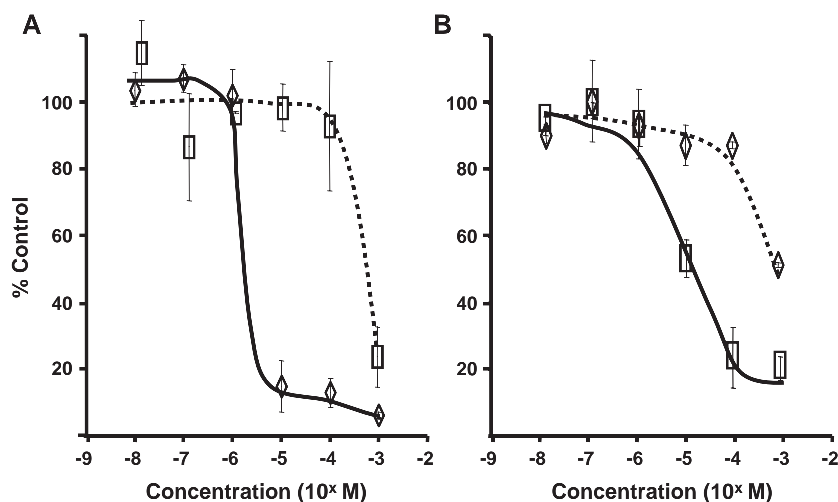


Fig. 2. (A) The percentage of control radiolabeled paclitaxel bound in tissue when in competition with unlabeled paclitaxel (□) and unlabeled rapamycin (◇). (B) The percentage of control radiolabeled rapamycin in tissue when in competition with unlabeled paclitaxel (□) and unlabeled rapamycin (◇).

between $10^{-4}$ and $10^{-3}$ M to displace significantly the labeled drug (data not shown). Tissue drug concentrations of $10^{-3}$ M have never been reported from paclitaxel or rapamycin-eluting stents.

### 3.2. Specific and general binding tissue domains

Transmural distributions of rapamycin (Fig. 3) and paclitaxel (Fig. 4) under conditions of specific and

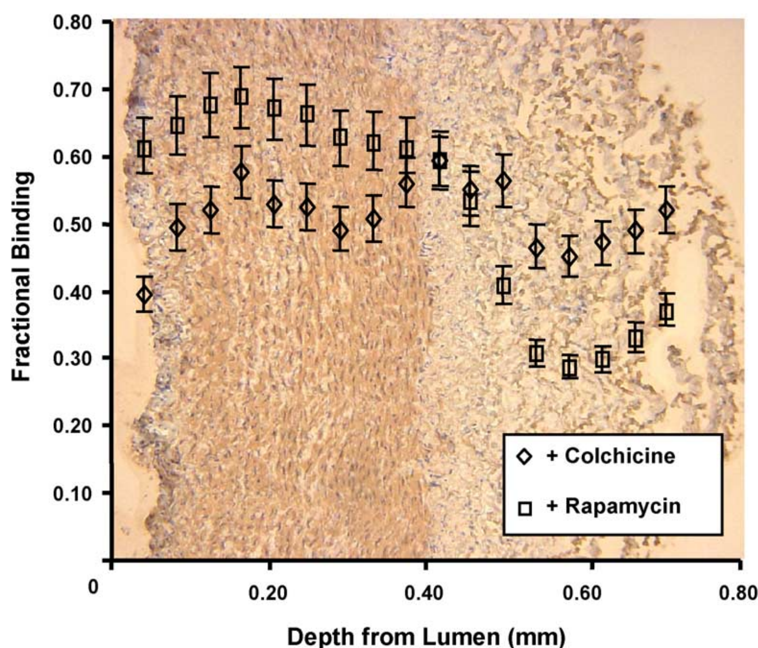


Fig. 3. Transmural equilibrium distribution of radioactive paclitaxel plus colchicine (◇) or rapamycin (□) in 0.040 mm thick bovine internal carotid tissue segments plotted over arterial tissue stained for tubulin protein (tubulin immunostain dilution 1 : 50). Each data point is normalized to no-competition profiles [1].

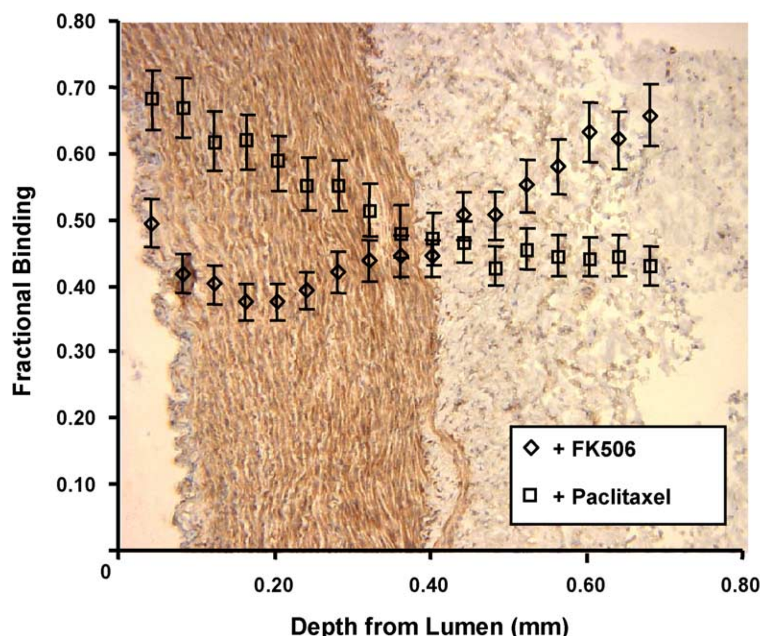


Fig. 4. Transmural equilibrium distribution of radioactive rapamycin plus FK506 (◇) or paclitaxel (□) in 0.040 mm thick bovine internal carotid tissue segments plotted over arterial tissue stained for FKBP (FKBP immunostain dilution 1 : 10) Each data point is normalized to no-competition profiles [1].

general displacement were plotted over tissue sections immunohistochemically labeled for each drugs' specific binding target and normalized to their control profiles. We have previously shown that rapamycin maintains a homogenous transmural distribution across the width of arterial tissue while paclitaxel distributes more variably, with relatively higher sub-intimal and adventitial binding and lower medial deposition [1]. We now differentiate between specific and general tissue binding. Colchicine, reduced normalized paclitaxel content by ~50% in a uniform manner across the media and adventitia, despite a seemingly higher density of tubulin staining paclitaxel-binding sites in the media. In contrast, rapamycin forced the paclitaxel transmural profile to drop precipitously from 70% in the media to nearly 30% in the adventitia, indicating increased blockade of general binding sites by rapamycin in the outer vessel wall. The rapamycin profiles followed similar trends to those observed with paclitaxel. When FK506 inhibited specific binding of rapamycin to tissue, medial drug concentrations decreased as expected. Paclitaxel reduced general binding in the adventitia relative to the media. For both rapamycin and paclitaxel, specific displacement of the drug correlates with a higher density of the specific protein binding target in the media, while general displacement is stronger in the adventitia where the specific binding site concentration is decreased. Together these profiles indicate that both specific and general binding are found in the media and adventitia but with stronger specific binding trends in the media for both drugs.

### *3.3. Local molecular specificity*

FKBP and tubulin binding assays were used to assess molecular binding specificity. Increasing concentrations of paclitaxel did not disrupt rapamycin binding to FKBP. These data imply that paclitaxel does not specifically bind to the rapamycin binding domain on FKBP and that paclitaxel and rapamycin do not associate with one another to decrease the amount of rapamycin available for binding (Fig. 5). As expected unlabeled rapamycin and FK506, which share the same binding domain on FKBP, do have a competitive effect. Similarly only colchicine, a tubulin depolymerizing agent, alters the polymerization state

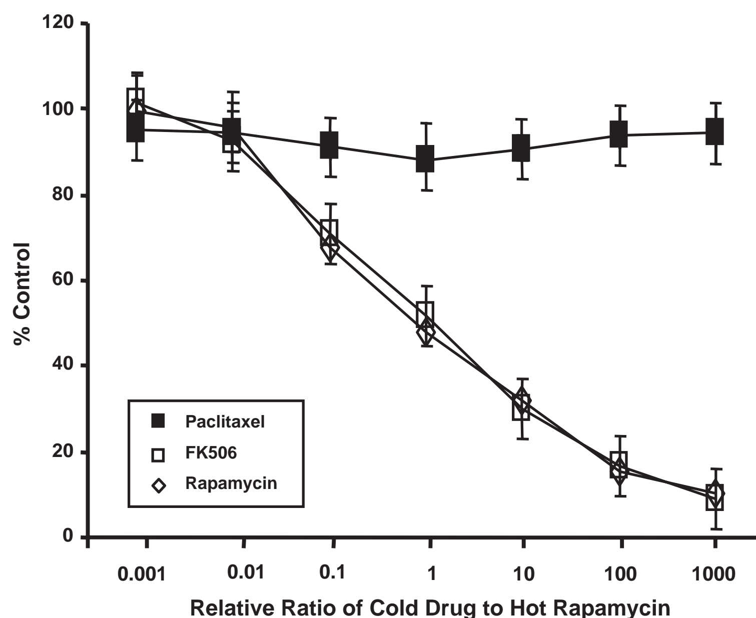


Fig. 5. Molecular specificity of FKBP binding. The percentage of radiolabeled rapamycin bound to human wild type FKBP plotted relative to no-competition controls for various unlabeled competitor drugs (indicated in the figure).

of tubulin in the presence of paclitaxel while rapamycin has no effect (data not shown).

### 3.4. Systemic to local competition

A number of drugs commonly administered to patients with cardiovascular diseases were simultaneously loaded in tissue with labeled paclitaxel or rapamycin (Fig. 6). Notably, insulin, captopril (ACE-inhibitor), atenolol and metoprolol (beta blockers) all significantly reduce arterial drug levels, likely by displacing labeled drug from general binding sites or offering alternative binding domains as may be the case with insulin. Salicyclic acid (aspirin), nifidipine (calcium channel blocker), hydrochlorothiazide (diuretic) and clopidogrel (anti-platelet) showed no significant reduction in drugs levels.

## 4. Discussion

DES are now the clinically dominate intervention for treating occlusive coronary vascular pathologies, and yet full mechanistic definition of their success and shortcomings remain elusive. Specificity in binding has emerged as a vital component of DES functionality with competition for binding space a critical factor in arterial drug uptake. We now report the potential competitive effects on DES tissue deposition by other compounds released from adjacent stents or circulating after systemic administration.

### 4.1. Local/local competition

Paclitaxel does not interfere with rapamycin binding to FKBP, and rapamycin does not perturb paclitaxel associations with tubulin. Each drug can displace the other from tissue but only at surrounding concentrations on the order of $10^{-3}$ M. Such high molar concentrations of drug may be relevant for systemically delivered therapies, but are not likely observed after elution from multiple proximate stents even if all of the drug on the stents would pool locally. In the context of overlapping stents, the question of rapamycin–paclitaxel drug competition remains an open and clinically relevant question. In certain scenarios, placement of two stents with different drug formulations may be favored over placing two identical devices. Additionally, a multi-drug combination stent, e.g., paclitaxel–rapamycin, may prove

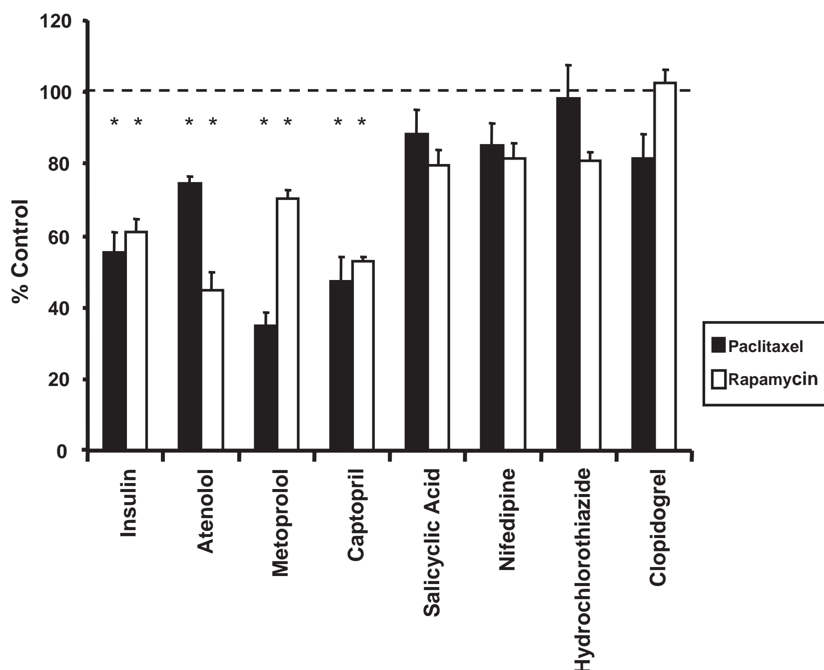


Fig. 6. Percentage of radiolabeled paclitaxel (■) and rapamycin (□) relative to no-competition controls in tissue after 24 h when simultaneously loaded with various unlabeled competitor drugs (indicated in the figure). (⋆) indicates a statistically significant difference ($p<0.05$ with a two-tailed Student's $t$-test) from the no-competition control. Standard deviations are plotted with the data set.

more efficacious than single drug therapies. Our investigations show no physiochemical contraindication to placing a pair of dissimilar DES in close proximity or placing two different drugs on the same stent. As more models of drug-eluting stents with different drug formulations emerge, these types of interactions may be therapeutically pertinent and should be considered in the evaluation of therapeutic viability.

Though DES has significantly reduced the number of restenosis cases, an appreciable proportion of patient population still manifest this condition [11–13]. No study has yet isolated the mechanism(s) of failure beyond identifying risk factors such as diabetes, previous interventions, lesion morphology, lesion dimension and vascular bed geometry [14]. Our models for specific protein binding inhibition with FK506 and colchicine demonstrate that variations in specific binding site availability significantly affects tissue uptake. Disease states such as diabetes or atherosclerosis may directly affect FKBP expression or tubulin polymerization, pathologically altering specific binding site availability and drug uptake capacity. In our model systems, blockade of general binding sites also reduces total tissue binding capacity. General binding sites throughout the tissue may serve as a reservoir for locally delivered drugs after the stent platform is depleted. Pathologic loss of general binding domains can also reduce overall tissue capacity.

### 4.2. Local/systemic competition

Systemically delivered compounds can maintain blood levels several log orders higher in concentrations than locally delivered drug tissue levels. The competitive displacing effects of systemically circulating drugs appear to be at the level of general binding. Since general binding sites reside in both the media and adventitia, systemic drug competition may reduce drug distribution levels throughout the vessel wall.

Heterogeneity exists between different screened compounds with regard to their overall effect on drug deposition and even between paclitaxel and rampamycin loading. The individual properties of each drug

may have specific properties which favor general binding site displacement.

## 5. Conclusion

Local drug delivery maintains great appeal for many pathologic conditions. In vascular systems at the level of local/local competition, rapamycin and paclitaxel do not appear to interfere with the other's specific binding to the therapeutically relevant tissue proteins. However, systemic drugs can displace local stent-eluted compounds from general binding sites and decrease tissue reserve capacity. As the applications and combinations of drug formulations for local and systemic drug delivery expand, the competition implications of concomitant delivery must be considered to optimize delivery methodologies.

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