PREFACE

Issues related to arterial vascular injury are central to the cardiovascular practitioner and research scientist alike. Whether acute (i.e., mechanically induced) or chronic (i.e., hypertension, atherosclerosis, and immune-mediated), vascular injury and the responses it elicits are leading causes of disease today, producing such acute ischemic syndromes as transient ischemic attacks, stroke, unstable angina pectoris, and acute myocardial infarction, as well as restenosis following percutaneous angioplasty or revascularization surgeries. The development of effective cardiovascular therapeutics to treat or prevent atherosclerosis and restenosis relies on preclinical research—both cell biological studies and observations and findings from animal models.

We have found that no one resource is available for a comprehensive presentation of animal models related to vascular disease. We hope that *Vascular Disease and Injury: Preclinical Research* will provide such a medium by presenting topics related to vascular injury in an organized and comprehensive fashion. Our approach is to present issues related to vascular disease and injury in five major areas: acute mechanical injury and vascular repair, models of arterial thrombosis, chronic atherosclerotic models, vascular disease in transplanted vessels, and vascular disease in models of systemic and pulmonary arterial hypertension. We have aimed to provide a "how-to" guide and have, therefore, worked to ensure that each chapter is highly practical by including equipment lists, current sources for animals, diet and reagents, schematic diagrams and, when pertinent, photomicrographs of sample histology.

In Part I of the book, Acute Mechanical Injury and Vascular Repair, Drs. Welt and Rogers review the widely used rabbit iliac artery models of balloon- and stent-induced angioplasty. Dr. Schwartz follows with a comprehensive presentation of the classic porcine overstretch stent model, emphasizing the relationship he has characterized between the degree of vascular injury and resultant neointimal thickening that follows. Dr. Carter extends this model into an atherosclerotic milieu. Drs. Nedelman and Rogers then apply central elements of these lower animal models to nonhuman primate experimental angioplasty and stenting, a burgeoning field suited to evaluation of human-targeted biologics. Since venous conduits are used extensively with high failure rates in coronary and peripheral bypass procedures, Dr. Dzau's group provides a chapter on pathologic responses in experimental models of arterial-venous grafting. Murine systems allow cardiovascular researchers to take advantage of key transgenic and knockout strains. Therefore, we have provided extensive material on recently described models of

acute and chronic vascular injury in mice. Dr. Lindner, who pioneered the use of mice in this field, discusses wire denudation and ligation models of neointimal thickening. Drs. Chen, Rogers, and Simon then describe a recently published model of arterial dilation and endothelial denudation that is accompanied by inflammatory cell recruitment and neointimal thickening. Drs. Eitzman and Westrick present a very interesting vascular photochemical model that has components of thrombosis, as well as neointimal thickening. Finally, Dr. Coller's group discusses their approach using a femoral wire injury model, a modification of the carotid wire denudation resulting in increased neointimal thickening.

In Part II, two chapters will focus on Models of Arterial Thrombosis. In the first, Drs. Fay, Parker, and Zhu use perivascular ferric chloride to induce arterial injury and thrombosis in the mouse carotid. These investigators have exploited this model to investigate the importance of plasminogen activator inhibitor-1 in modulating endogenous fibrinolysis. Finally, Dr. Folts provides a comprehensive overview of his animal preparation for studying in vivo platelet activity and platelet interactions with damaged arterial walls. This model has been instrumental in the clinical development of therapeutics for acute ischemic syndromes and percutaneous coronary interventions.

Part III focuses on Chronic Atherosclerotic Models. Drs. Palinski, Napoli, and Reaven provide an in-depth overview of mouse models of atherosclerosis, in particular the apolipoprotein E (ApoE) and low density lipoprotein (LDL) receptor knockouts. Drs. Aikawa and Libby then present their work regarding progression and regression of atherosclerosis using the classic hypercholesterolemic rabbit model. Finally, Drs. Nicolosi and Kritchevsky present the use of higher animals, including nonhuman primates, for preclinical research in atherosclerosis.

Part IV of the book concentrates on Vascular Disease in Transplanted Vessels. Drs. Shi and Hoover discuss the use of a murine carotid loop model of transplant disease that has been helpful in elucidating the important role of proteases, such as plasminogen, in transplant-related vascular disease. Dr. Mitchell then follows with an overview of heterotopic heart transplantation in the mouse. His group has used this model to study the role of cytokines and immune co-stimulatory molecules in parenchymal rejection and accelerated graft arteriosclerosis. The concluding chapter in this section by Drs. Chen and Adams presents exciting material regarding hyperacute vascular rejection in pig-to-primate xenotransplantation.

The next set of chapters in Part V concentrates on Vascular Disease in Models of Arterial Hypertension. Dr. Baumbach examines methods for investigating cerebrovascular disease in experimental systemic hypertension. Two chapters are devoted to pulmonary hypertension. In the first, Dr. Rabinovitch provides an in-depth discussion of monocrotaline-induced pulmonary hypertension. She focuses on the cellular and molecular biology of pulmonary vasculopathy, integrating dynamic interactions between smooth muscle cells, extracellular matrix, and the

endothelium. This leads into the chapter by Drs. Meyrick and Tchekneva on chronic pulmonary hypertension in the hypoxic rat and in the sheep following continuous air embolization.

The final section, Part VI, provides an essential foundation in Animal Care and Tissue Processing and analysis. Dr. Marini discusses veterinary issues and anesthesia options, addressing all species covered elsewhere in the book, from mice to nonhuman primates. Key points regarding survival surgery, choice of anesthetic, and analgesia are included. Histopathologic methods are then discussed by Drs. Seifert, Rogers, and Edelman. This chapter provides the "basics" for tissue harvesting and fixation, and histology methods for routine immunology and electron microscopy.

The topics we have chosen to include in *Vascular Disease and Injury: Preclinical Research* are not meant to be all inclusive and, undoubtedly, a few areas have not been covered. We have simply tried to show the range and breadth of animal models that have been useful in translational cardiovascular research. It is important to end this discussion on a cautionary note. The track record of animal models of vascular repair after injury, as predictors of human responses, is poor. Myriad agents have been proven effective in one or another model, only to fail clinical scrutiny. This fact means that for each experimental approach, by any of the models described in this book, the purpose of research must be to further mechanistic understanding, not to recapitulate human disease in an experimental animal.

In closing, we must acknowledge the tremendous efforts of our administrative assistant, Paula McColgan, the series editor, Dr. Christopher Cannon, and the staff of Humana Press. We are indebted to Drs. Eugene Braunwald, Victor J. Dzau, Thomas W. Smith, and Peter Libby for encouraging and supporting our clinician-scientist careers. Dr. Rogers would like to thank most deeply his mentors in the study of vascular injury and repair, Drs. Morris Karnovsky and Elazer Edelman, and to dedicate this book to his wife Nathalie and three children, Camille, Genevieve, and Charles. Dr. Simon would like to honor his mentor in life and medicine, Dr. Norman M. Simon, and to dedicate this book to his wife Dr. Marcy Schwartz and three children Benjamin, Maxwell, and Aaron.

Daniel I. Simon, *MD*
Campbell Rogers, *MD*

2 The Porcine Model of Coronary Restenosis

Robert S. Schwartz, MD, Birgit Kantor, MD, and David R. Holmes Jr., MD

CONTENTS

INTRODUCTION

Human coronary restenosis remains an elusive problem, and a major limitation of all percutaneous interventional coronary revascularization procedures, despite intracoronary stenting *(1–9)*. Restenosis has recently gained even greater importance, since trials comparing PTCA with coronary bypass surgery (BARI, EAST, CABRI) suggest that angioplasty is comparable therapy for cardiac events and symptoms, but the two differ strikingly regarding the need for repeat interventions and cost *(10–12)*. Restenosis lies at the center of these problematic differences.

A wide spectrum of pharmacologic strategies have demostrated either complete failure, or at best equivocal success *(13–27)*. New devices have also failed to show substantial effect *(28)*. The incidence, clinical time course, and angiographic correlates of coronary restenosis have been well described, yet a limited understanding of its pathophysiology has prevented the formulation of

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Table 1
Clinical Problems Involving Exuberant Neointimal Hyperplasia

Small-diameter vascular grafts
Prosthetic grafts
Vasculitis
Transplant coronary artery disease
Atherosclerosis

a truly effective therapy. Only recently has vascular brachytherapy with β or γ radiation suggested that neointimal hyperplasia may be limited.

While many animal arterial injury models have been developed and extensively studied to test potential therapies, a limited knowledge of the relevance of such models to human restenosis poses a major drawback. These models have been used to test preclinical therapies, and to provide a better understanding of the pathophysiology of the restenosis problem. Studies using such models have provided a framework for a better knowledge of the arterial response to injury *(29–31)*.

Published results from many animal studies often fail to translate to clinical trials, resulting in confusion about the models, restenosis mechanisms, and potential solutions. However, in most instances, a careful review and consideration of such studies frequently reveals that the interpretation of the results, and not the models themselves, have failed. In general, the porcine model of restenosis seems practical, and substantially representative of human remodeling and neointimal formation. We must formulate a better understanding of this useful model to determine when and how far to apply it in understanding the restenosis problem.

Restenosis in its simplest form is the healing response following arterial injury caused by revascularization *(32–38)*. It is commonly attributed to several factors, including acute and chronic remodeling, *(39–42)* thrombus at the injury site, medial smooth-muscle-cell (SMC) migration and proliferation, and extracellular matrix production *(43–49)*. In these times, when coronary stent placement is ubiquitous, remodeling at the angioplasty site is minimized. However, the stent itself enhances neointimal hyperplasia, reducing the problem to understanding and limiting neointimal thickening *(50)*. Neointima plays an important role in many arterial diseases (*see* Table 1).

PORCINE CORONARY ARTERY INJURY MODELS

The coronary arteries of domestic crossbred pigs respond in a very similar fashion to human coronary arteries after sustaining deep injury *(51)*. A hyper-

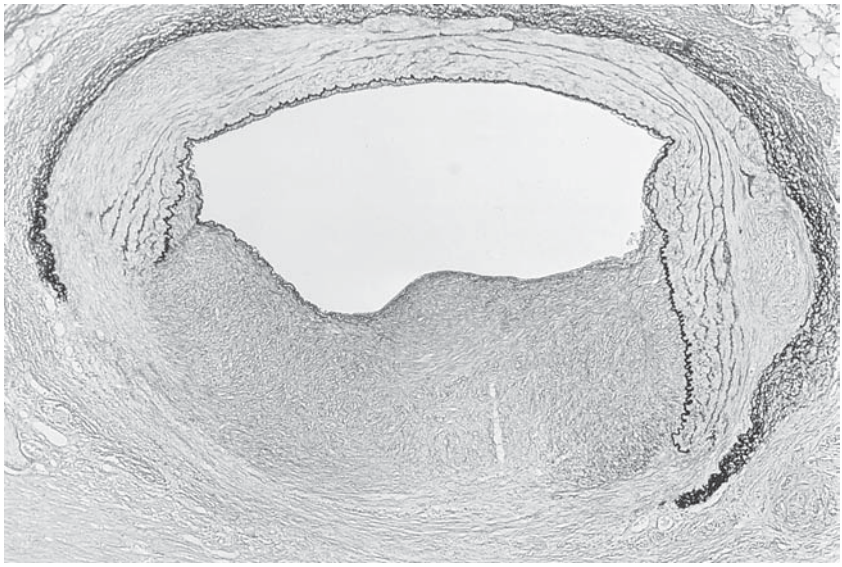

Fig. 1. Photomicrograph of a porcine coronary artery 28 d after oversized balloon injury. Not all wires penetrated into the vessel media. The balloon lacerated the media and created a large dissection *(bottom)* that filled with neointima. The neointima grows only at sites of internal elastic lamina and medial rupture. The amount of neointimal formation is variable, and in general is proportional to the size of the fracture length. A typical response index is intimal area/fracture length. Elastic van Gieson's stain, magnification × 10.

cholesterolemic diet produces lesions which are histopathologically identical, but more severe than those produced by standard laboratory diets *(52)*.

The carotid arteries are typically used for arterial access in this model, although the femoral arteries may also be used without difficulty. Standard human coronary-guide catheters and curves for human coronary angioplasty fit the porcine aortic root well (20–40 kg animals) for engagement of the left main or right coronary arteries.

Severe mechanical arterial injury is done to the coronary arteries either by a coronary angioplasty balloon alone, *(30,53,54)* or by delivering an oversized metal coronary stent to the artery for chronic implant. Both methods create an injury that results in a thick neointima within 20–28 d (*see* Figs. 1–3). The histopathologic features of this neointima are identical to human restenotic neointima (Fig. 4), and the neointima is often voluminous enough to cause relevant luminal narrowing.

Specimens from balloon-injured vessels without stents typically show a single laceration of media, filled at 28 d by a variable amount of neointima. Oversized stent placement in arteries show multiple injuries in each section.

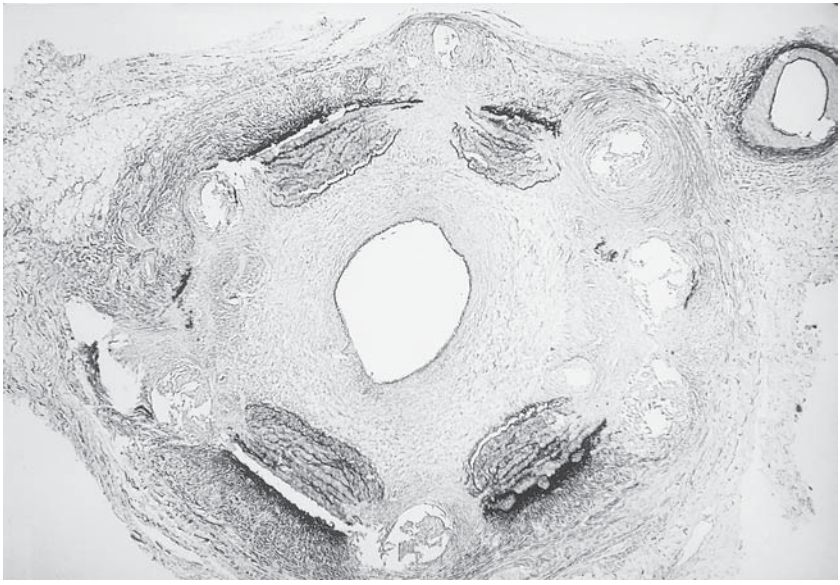

Fig. 2. Section showing severe injury in a normal porcine coronary artery at 28 d after coronary artery injury. All wires have produced severe damage, as evidenced by voluminous neointimal thickening at all sites circumferentially around the lm. The lm is markedly compromised by this injury. Elastic van Gieson's stain, magnification × 10.

Each injury site is characterized in the porcine oversized stent-injury model as a mean injury score (Table 2) that is ordinally proportional to injury depth *(53,54)*. The amount of neointimal thickening is directly proportional to this score (Fig. 5). This permits creation of an injury-response regression line that can be used to quantitate the response to potential therapies *(55)*.

An interesting consideration is whether neointimal formation resulting from injury by balloon alone differs from that caused by oversized stents, and several considerations are important to answering to this question. The first is whether the stent alters the mechanism of neointimal formation. Neointimal thickness is strongly related to the depth of injury in the stented injuries—an observation which has important implications. At low or zero levels of arterial injury, neointima at stent-wire sites is quite thin—essentially the same as that of "appropriately sized" stents. It is only when stent wires fracture the internal elastic lamina, lacerate media, or perforate through the external elastic lamina that neointimal thickness grows substantially to the point of creating macroscopic stenoses. This set of observations suggests that it is the *injury* from the stent wires, rather than the wires themselves, that is responsible for neointimal generation. The stent is thus a means of reliably producing injury to the arterial

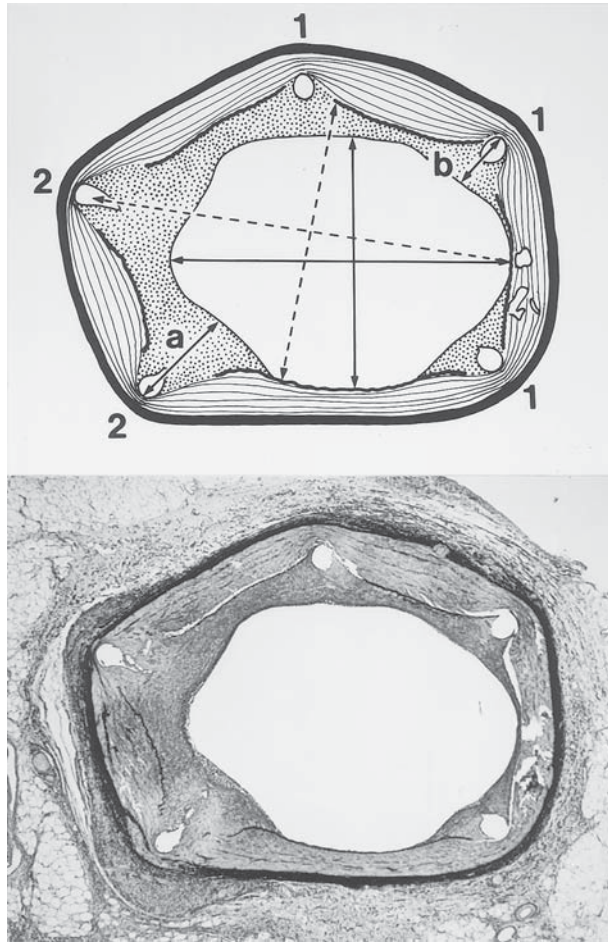

Fig. 3. Photomicrograph of a porcine coronary artery 28 d after severely oversized coil injury. Not all wires penetrated into the vessel media. In this section, the two coils at the bottom of the vessel lacerated the media and resulted in substantial neointimal thickening. Conversely, the farthest right wire did not, and less thickening resulted. A short segment of vessel media at the bottom-most portion of the figure is entirely normal, without any neointima, although this segment was stretched by the balloon. This normal-appearing segment has the farthest distance between any coil wires. The top image shows the method of quantitating injury produced by the stent wires. The Elastic van Gieson's stain, magnification × 10.

wall. When the stent itself is not a cause of injury, it does not produce substantial neointimal thickening. Evidence from studies with rabbit femoral arteries indicates that oversized, injurious stent wires provide a strong, prolonged stimulus to mitosis in the intima of the vessel. It is also clear that the stent metal

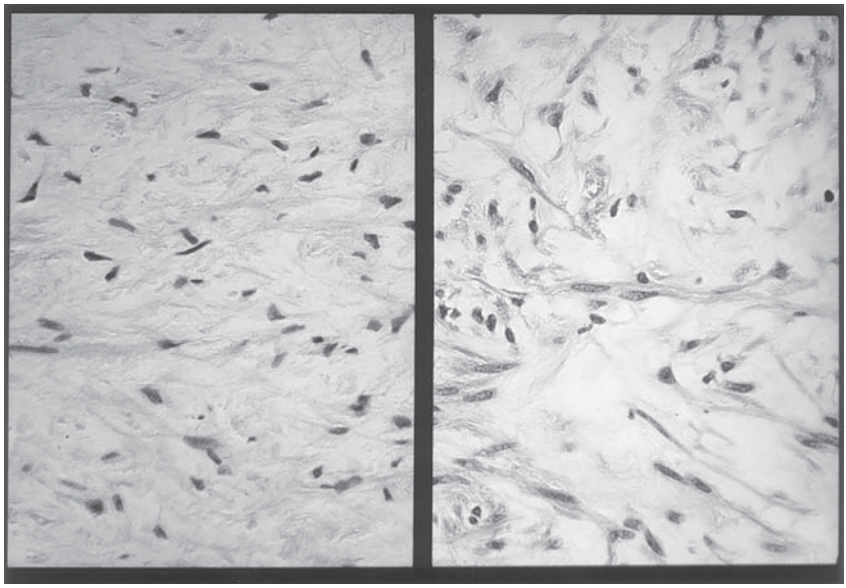

Fig. 4. High-power side-by-side comparison of a representative sample of human restenotic neointima *(left)* and tissue from the porcine restenosis model *(right)*. The character of cells and proportion of ground substance is histopathologically identical. Hematoxylin Eosin stain, magnification × 300.

Table 2
Ordinal Arterial Injury Score

Score	*Injury*
0	Internal elastic lamina intact; endothelium typically denuded; media compressed but not lacerated
1	Internal elastic lamina lacerated; media typically compressed but not lacerated
2	Internal elastic lacerated; media visibly lacerated; external elastic lamina intact but compressed
3	External elastic lamina lacerated; typically large transluminal lacerations of media; coil wires sometimes residing in adventitia

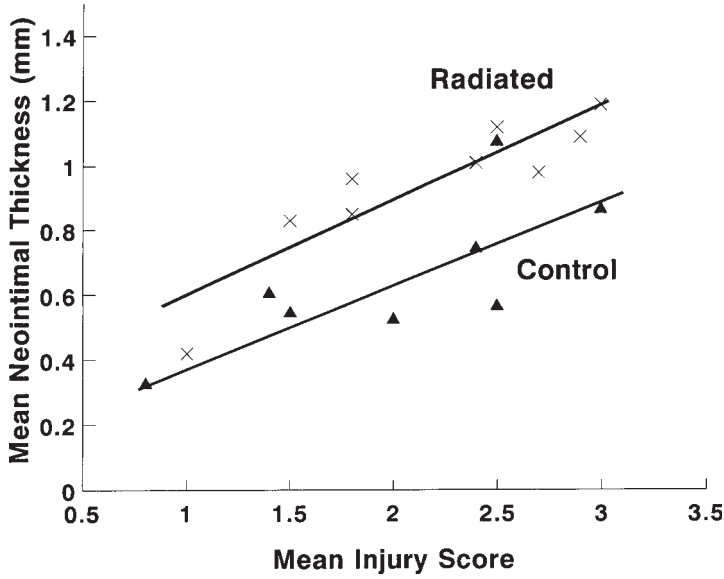

Fig. 5. Regression lines of mean neointimal thickness vs mean injury score for porcine 26 coil-injured coronary-artery segments. The two regression lines are from a study comparing external-beam radiation with control, no radiation. The external radiation exacerbated the injury, and worsened the neointimal thickening. This is shown by a parallel regression line, but with a larger y-intercept.

causes essentially no foreign-body reaction, since many studies have shown little or no chronic inflammatory cellular response at wire sites (i.e., no giant cells). Most importantly, the stent in this model assumes even greater importance when considering that a majority of patients receiving angioplasty also receive stents.

One reason for the greater neointimal thickening with oversized stent placement is that typically, five or more injury sites result in a localized region around the vessel circumference, each generating neointima. This type of injury pattern differs from the inflation-only injuries, where a single large dissection is typical (Fig. 1). This injured location is the site of neointimal development.

In the oversized stent model, quantitation of vessel injury is facilitated by the discrete stent injury points, and the exact size and extent of injury can be measured and compared directly with the neointimal thickening response using regression methods. A similar proportional response between injury and neointimal thickness has been shown by Bonan for the inflation-only injury method *(56)*. This consistency with the injury-neointimal-thickness response found for the oversized stent injury method is reassuring; the neointima of both models is likely formed by similar mechanisms. It is possible that thrombus volume dif-

fers at the injury sites for inflation-only and oversized stent-injury models. This may be caused either by the stent itself, or the increased injury present in the vessel wall that in turn causes increased thrombus deposition. It is likely that increased thrombus is partially responsible for the greater amount of neointima occurring in the stented model. The mechanisms of healing—whether from balloon inflation only or oversized stent—are the same. These photomicrographs, (Fig. 1) from a balloon-only coronary artery injury, show a typical single medial dissection beginning to heal. Thrombus is present early, and is heals from the *luminal* side toward the adventitial surface. A thin cap of SMCs is present on the luminal surface of the thrombus. This finding should not be surprising—it would be unusual to find stented arterial injuries healing through different mechanisms than inflation-only injuries. Recent findings from irradiated arteries in both human patients and pigs suggest that mural thrombus attached to and covering the stent struts and the injury site is the earliest response in the healing process. In cases of irradiated vessels, healing is halted and only such layers of thrombus are present, without cellular organization.

The oversized stent and inflation-only porcine coronary-injury models are thus quite comparable. Reliability of lesion generation depends primarily on the operator's ability to cause enough arterial injury to generate neointima in either model, but not so much that acute vessel thrombosis occurs with resultant animal death. This finite incidence of thrombosis is considered a problem by investigators, but is in fact a representation of stent thrombosis that occurs in human patients.

Quantitation of vessel injury and the neointimal thickening response is facilitated in the oversized stent model, because discrete injury points are observed and quantitated. The differences and similarities of these two models are summarized in Table 3. The importance of proper, blinded quantitation cannot be overemphasized in this context.

ANIMAL RESTENOSIS MODEL TESTING: DIVERGENT RESULTS FROM CLINICAL TRIALS

Many pharmacologic agents have been tested in the animal models described above, and representative results are summarized in Table 4. These data indicate that many agents are effective in animal models, yet these same agents are *ineffective* when tested in human clinical trials. Examples are antiplatelet drugs, *(27,57–61)* anticoagulants, *(62)* calcium-channel blockers, (*63,64)* angiotensin converting enzyme inhibitors, *(15,65)* and antiproliferatives *(13)*. The disparity of results between animal-model research and clinical trials has led to skepticism about the validity of animal models in restenosis research.

Table 3
Comparison of Oversized Stent and Inflation-Only Porcine Model

	Oversized stent	*Balloon injury only*
Number of injury sites	Multiple	Single
Size of injury sites	Smaller, constant	Larger, variable
Injury quantitation	Easier (injury score)	More difficult (fracture length)
Response variables	Neointimal thickness Neointimal area Lumen area Injury-NI thickness Regression	Intimal area (IA) Fracture length (FL) Quotient IA/FL
Neointimal response to injury	Proportional	Proportional
Thrombus at injury site	Present	Present

Many therapies effectively limit proliferation and migration in rat carotid arteries. Why do the results of so many animal studies not reflect those seen in clinical trials of the same agents? A number of interpretations explain this observation. One consideration is that the rat carotid model is the oldest. More agents have been tried, and thus more have been found successful in this model. The rabbit iliac model has also been extensively studied and tested. Since the porcine models are newer, fewer agents have been tested and found effective. Are the mechanisms of neointimal formation different among these animal models and in human patients? Are other factors in the models themselves or their analysis methods responsible for the discrepancies? The answers to these questions are unknown, but are essential for developing solutions based on animal-model data.

In the rat carotid model, proliferation of SMCs has been documented in detail *(66–68)*. Yet neointimal volume in these injured arteries is small, and rarely causes arteriographically detectable luminal stenoses. The porcine model also shows cellular proliferation, but hemodynamically significant stenoses regularly occur. Are the pathophysiologic mechanisms different across species? Strong teleologic arguments must be raised against the hypothesis that the arterial response to injury occurs differently across species. The apparent disparity in animal-model results must be examined if they are to be reconciled by a unifying hypothesis of restenosis pathophysiology.

Table 4
Porcine Coronary Model

Agent	*Efficacy*	*References*
Angiopeptin	++	*95–97*
Lovastatin	+/-	*98, 99*
Hirudin	+	*100, 101*
Methotrexate	N	*102*
Probucol	++	*103*
Trandolapril/Captopril	N	*104*
Enalapril	N	*105*
AII Inhibition	N	*106*
X-Irradiation	N/++	*73, 107–117*
Endothelin Inhibition	+/-	*118*
Antisense: CDC2/PCNA	N	*119*
Vitamins C/E	N	*120*

Key: ++ Effective in Neointimal Reduction
N Not Effective

TRANSLATING RESULTS OF ANIMAL MODELS TO CLINICAL TRIALS

The porcine coronary models using either the stent or overstretch injury alone have increasingly become the standard by which potential restenosis therapies are applied. In the past, negative trials in the pig have corresponded to negative clinical trials, suggesting that this model has specificity. Since there were few or no therapies available that showed positive results in human patients, the effective sensitivity of the model was uncertain. Recent clinical trials suggest that ionizing radiation may limit neointimal hyperplasia in human patients *(69–72)*. Interestingly, the pig model showed that external-beam radiation was not only ineffective against neointima—it actually *stimulated* growth *(73)*. More recently, other investigators have examined intravascular radiation and found this modality effective against neointima. Interestingly, subsequent clinical trials suggest the efficacy of intravascular radiation in human patients. This seminal observation—if demonstrated with subsequent larger randomized trials—will add useful data to our understanding of precisely how the porcine model will translate when applied to human patients. Specifically, the multiple methods of assessing efficacy in the pig coronary (percent stenosis and reduction, neointimal thickness, remodeling) (*see* Fig. 6A–C) will be considered, and the best correlate of human data determined. Subsequent new or modified therapeutic modalities may then be tested to rapidly converge on the best treatments for the problem.

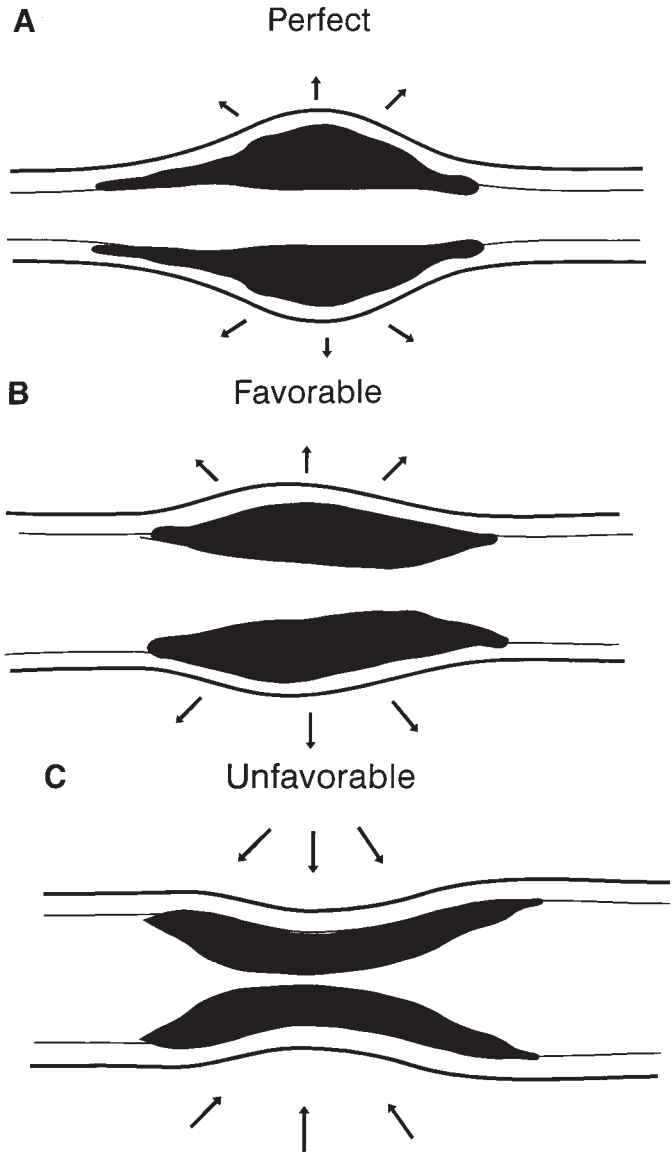

Fig. 6. Schematic representation of three types of remodeling: perfect, favorable, and unfavorable. **(A)** In perfect remodeling, the artery expands its diameter perfectly to compensate exactly for the vol of neointima that grows. The lumen is not compromised in this situation. **(B)** This figure shows favorable remodeling. In this case, the artery cannot perfectly remodel, but is able to partially expand in an attempt to accommodate neointimal thickening. The lumen is only partially compromised by the neointimal thickening, while expanding outwardly and incompletely. **(C)** In unfavorable remodeling, the artery either does not expand at all, or actually contracts. The artery develops a severe stenosis as a result.

The thrombotic response to arterial injury may differ substantially across species. In the rat carotid model, a thin layer of platelets accumulates at the endothelial denudation site. However, significant fibrin-rich thrombus is virtually never found in this model. Conversely, in the rabbit iliac model, macroscopic thrombus does occur, as characterized in a preliminary report *(74,75)*. In the porcine carotid and coronary models, fibrin-rich mural thrombus also plays a significant role in the response to injury. In the coronary arteries, fibrin-rich thrombus provides a framework for colonization by medial SMCs. This foundation eventually forms the organized neointima, a mechanism also suggested in the rabbit. The question of mural thrombus vol and its relation to eventual neointimal vol is critical, and is under investigation. Differences in mural thrombus vol formed in the days and weeks following angioplasty could govern the occurrence of restenosis, as suggested by the rabbit and porcine models. Differences of native thrombolytic potential across species might partially explain differences in mural thrombus. The distinction between "proliferation" and "thrombus" may be blurred, since proliferation may be occurring within thrombus. The rat carotid artery may not generate substantial neointimal vol (and macroscopic stenoses), because it does not form macroscopic thrombus. This suggests an explanation for agents effective in the rat carotid model, yet ineffective in human clinical trials. These agents might be very effective in reducing SMC migration and proliferation, yet exhibit little effect on chronic mural thrombus deposition. Only a part of restenotic neointimal formation may be addressed by these strategies, resulting in clinical failures.

REASONS FOR THE FAILURE OF ANIMAL MODELS TO PREDICT CLINICAL RESULTS

Questions remain about why certain therapeutic strategies which successfully inhibit neointima in some animal models fail to predict clinical trial results. Several potential explanations exist for these discrepancies.

There is still uncertainty regarding how doses of pharmacologic agents given to rodents and other small animals translate to comparable human doses. Two examples from the literature are noteworthy. Studies have shown that Angiotensin Converting Enzyme (ACE) inhibition effectively limits neointimal formation in the rat *(76–81)*. In a key study, *(77)* the common carotid arteries of rats were denuded of endothelium in the usual fashion, and animals were treated with either captopril 100 mg/kg or cilazapril 10 mg/kg body weight per d beginning 6 d before arterial injury and continuing until the time of euthanasia. An impressive reduction in the percentage of neointimal coverage of the internal elastic lamina was found in both drug treatment groups (42 ± 11% captopril treated vs 111 ± 10% control, and 35 ± 9% cilazapril treated vs 93 ± 5% control). This important study provided the stimulus for two large, well-executed

clinical trials of cilazapril in Europe (MERCATOR) and the United States (MARCATOR).

Both clinical trials showed this agent had essentially no impact on restenosis *(65,82)*. The highest cilazapril dose used in MARCATOR was 20 mg/d for 24 wk. In a 70-kg patient, this dose corresponds to 0.29 mg/kg body weight, or 2.5% of the dose reported effective in rats on a body weight basis. In patients, even 20 mg/d was high, because many patients were intolerant as a result of orthostatic hypotension and other side effects. A marked discrepancy thus existed between the effective dose in rats compared to humans. Furthermore, the most effective regimen in rats involved 6 d of drug pretreatment before injury. This pretreatment regimen was not used in either the MERCATOR or MARCATOR trials.

A similar situation is found in a study of colchicine in the rabbit iliac artery model *(83,84)*. Colchicine was administered to rabbits at either 0.02 mg/kg-d or 0.2 mg/kg-d. The endpoints of this study were angiographic luminal diameter. Neointimal thickening in the control group changed from a mean of 1.7 ± 0.3 mm immediately following angioplasty to 0.6 ± 0.4 mm. In the group receiving colchicine (0.2 mg/kg), mean luminal diameter was reduced from a mean of 1.7 ± 0.3 mm following angioplasty to 1.1 ± 0.6 mm. In the 0.02 mg/kg-d colchicine group, mean luminal diameter dropped from 1.7 ± 0.3 mm following angioplasty to 0.9 ± 0.5 mm—a result not statistically different from control. In the high-dose colchicine group, the incidence of restenosis was reduced by 50%. However, studies of colchicine in patients have shown no evidence of clinical benefit when used in doses of 1.2 mg/d or 1 mg/d, with angiography or exercise thallium scintigraphy as endpoints *(26,85,86)*. The equivalent doses in a 70-kg human were 0.01 mg/kg-d, or only 5% of the most effective dose in rabbits. The side-effect profile of colchicine is well-known. Colchicine doses as high as 0.2 mg/kg-d in patients would be impossible to achieve without severe side effects.

In the pharmacology of drug testing across sppeies (including human patients), dosing is generally begun at comparable weight-adjusted (mg/kg) levels. It is possible, but unlikely, that the high doses used in rats and rabbits were comparable in efficacy to the doses used in the clinical human trials.

The normal coronary artery of a young rat, rabbit, or pig differs markedly from the atherosclerotic coronary artery of an older human patient. The arteries of these animal models—even those of the hyperlipidemic rabbit (developing over a period of 4 wk instead of decades as in humans)—do not show densely fibrous and acellular plaques with ulceration, calcification, thrombosis, and hemorrhage into the vessel wall. The impact of this atherosclerotic environment on restenosis is unknown. Whether the use of models that produce atherosclerosis will have advantages over nonatherosclerotic models is unknown. Yet, considering that restenosis is a response to arterial injury, there are only minimal differences in healing time as a function of age.

The positive relationship between arterial injury and neointimal thickness has been documented in the porcine coronary and carotid arteries. Clinical patient studies are emerging that also support a proportionality between increased vessel injury during revascularization and increased neointimal thickness. This proportional response in patients must be inferred only indirectly, since arterial injury cannot be assessed angiographically. Surrogate parameters for vessel injury include balloon:artery ratio, severity of initial stenosis (i.e., more severe stenoses undergo a larger relative dilation), acute complications, and the size of the initial lumen immediately following angioplasty. Most have correlated with increased restenosis risk in clinical studies *(87,88)*. A major advantage of histopathologic assessment in animal models is that vessel injury can be directly and semiquantitatively assessed. If a proportionality exists between depth of injury and neointimal response in animal models other than the porcine coronary model, it might be of substantial benefit in the models. Typically, artifact results when vessel injury is not accounted for as a covariate in animal studies, since conclusions regarding differences in efficacy might result from differences in injury among the treated and control groups.

The methods used to determine biologic response play a pivotal role in the outcome of any study. The most quantifiable and tangible outcome of clinical trials is quantitative coronary angiographic measurement of absolute lm size, or percent luminal stenosis. The issue of defining restenosis has been fully explored in published studies *(89)*. Restenosis rates using quantitative coronary angiography vary widely even within the same patient data set, depending on the definition used.

In animal-model studies, quantitative histopathologic measurements are generally the endpoints used to determine efficacy. Much quantifiable information is available from microscopic examination of histopathologic specimens. The area of neointima, media, and residual lumen size can be measured precisely and compared across treatment groups using digital microscopic methods.

The study of cilazapril in rats would have reported a negative conclusion if the accepted angiographic criteria of 0.72 mm minimum luminal diameter change had been applied to the histologic lumen diameter data. Data from this study were analyzed using three measurements: neointimal area, the quotient of (neointimal/medial area), and percent coverage of the internal elastic lamina by neointima. Since the media is typically 50 μ in the rat, neointimal formation is typically 50–100 μ thick. Although the inhibition of neointimal thickness by cilazapril was 80%, the *absolute* inhibition was only 90 μ (0.09 mm). Inhibition of neointimal thickness must be at least 0.36 mm to be minimally detectable using angiography *(90–93)*.

In another example, lovastatin was studied for its ability to reduce neointimal thickening in the nitrogen-desiccated hypercholesterolemic rabbit iliac artery, using angiographic endpoints *(94)*. The mean angiographic arterial diameter in

the control group immediately following angioplasty was 1.73 mm. At follow-up it was 0.91 mm—a difference of 0.82 mm. In the lovastatin-treated group, the immediate postangioplasty result was 1.44 mm, decreasing at follow-up to 1.16 mm—a change of 0.28 mm. Although statistically significant, these changes (1.82–0.28, or 0.54 mm) would not be discernable within angiographic definitions of clinical trials. While the data from this study clearly demostrate a modestly beneficial effect from lovastatin, the identical angiographic result in a human trial would be interpreted as having no effect.

The assessment of histopathologic efficacy is important, and should be performed in all animal studies. However, to better predict results in human trials when performing animal studies, microscopically planimetered minimal luminal diameters and percent stenoses should be measured. These measurements more accurately represent surrogate parameters for what would be found in a human angiographic restenosis trial. Variability of efficacy measurement may thus be a major factor in explaining why successful animal-trial results have not translated to clinical efficacy.

CONSISTENCY AMONG ANIMAL RESTENOSIS MODELS: A UNIFIED APPROACH

Many similarities exist among the animal restenosis models. Neointima forms through SMC migration, proliferation, and matrix synthesis in response to injury in all models. How can the apparent differences be reconciled?

The primary differences among animal models lie in the *volume* of neointima from a certain amount of arterial injury. As noted previously, studies of neointimal formation over time in both porcine and rabbit models suggest that mural thrombus at the injury site is a major determinant of neointimal vol. The healing process occurs from the *luminal side outward toward adventitia*. Smooth-muscle-cell migration from nearby medial sites has been documented in the porcine model, both for balloon inflation-only injuries and oversized stent injuries.

CONCLUSION

The importance of using analysis methods comparable to clinical trials (angiography, intravascular ultrasound) should be applied to animal trials. The many response variables to injury for the artery should be studied to determine which can best predict results in human trials. Different data analysis methods may play a major role in the variability of studies. Coronary angiography is the "gold standard" in patients against which all treatments will eventually be tested; thus arterial lm size (absolute and relative or percent stenosis) must be evaluated when analyzing data from animal-model studies.

The importance of using similar drug doses and timing for animal models and clinical trials cannot be overstated. Effective agents may have already been tested in the wrong doses or timing, with false-negative results. If concentration is a problem because of side effects, local delivery to the angioplasty site may be considered.

The variability of restenotic neointimal formation in different species is substantial. At either end of the spectrum of neointimal vol, species should be carefully analyzed for clues explaining why some species generate very little neointima following coronary-artery injury. The current animal models may be far more alike than at first apparent from the divergent results in published studies.

A stratified approach to testing potentially effective agents in multiple animal models should be implemented *before* clinical trials to minimize the possibility of negative results. Agents may be screened in the rat carotid-artery model before testing in other animal restenosis models and before human trials.

While there may be no perfect animal model for human restenosis, modeling a biologic process should be conducted to first understand the mechanisms of that process, followed by formulating and testing therapeutic strategies based on well-founded hypotheses. Strategies should be designed and tested to verify or refute these individual hypotheses. For restenosis, this process has been reversed: in the rush to solve the problem, understanding the biologic process is far from complete. Numerous pharmacologic agents and new device technologies have been tested in models without firm hypotheses for mechanisms. The limitations of these models are poorly understood, because of the markedly divergent results in human studies.

A solution to restenosis will result from the continued, meticulous study of neointimal formation in many models, leading to a full understanding of the limitations of the models and preventing erroneous conclusions from those models when applied to clinical trials.

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Additional Reading and Background

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