INTRAPERICARDIAL THERAPEUTICS AND DIAGNOSTICS (IPTD):

POTENTIAL ADVANTAGES

RECENT ADVANCES

EXPERIMENTAL DIRECT THERAPY OF CARDIAC DISEASES AND ARRHYTHMIAS

DAVID H. SPODICK, M.D., D.Sc., FACC
Guest Editor

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Intrapericardial Therapeutics and Diagnostics (IPTD): Potential Advantages, Recent Advances, Experimental Direct Therapy of Cardiac Diseases and Arrhythmias

DAVID H. SPODICK, M.D., D.SC., FACC, Guest Editor

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ARTICLES IN BRIEF

Original Contributions

I-2 Microphysiology of the Pericardium in Relation to Intrapericardial Therapeutics and Diagnostics
D. H. Spodick, M.D., D.Sc., FACC

Intrapericardial delivery of therapeutic agents for pericardial diseases has long been available in the presence of excess pericardial fluid. Most patients with myocardial and coronary disease have no such excess so that their direct treatment requires pericardial access, for which a new instrument has succeeded in animals with induced infarctions, coronary lesions and arrhythmias. Nitric oxide donors, calcium-avid drugs, antibodies, angiogenic agents (pharmacologic coronary bypass), and hypothermic solutions have been instilled intrapericardially, and even iontophoresis has been used; gene therapy is also promising. Intrinsic pericardiogenic substances potentially may be stimulated for comparable purposes.

I-4 Function of the Normal Pericardium
R. Shabetai, M.D.

Using the pericardial space as a depot for cardiovascular drugs necessitated the development of techniques to instrument the normal pericardium. These techniques have the potential to be exploited to further our understanding of pericardial function. Pericardial physiology includes intrapericardial pressures and their relation to ventricular function and the ambient (intrathoracic and intrapleural) pressures, yielding transmural cardiac pressures and responses, respectively, during the respiratory cycle. A continuing point of discussion involves appropriate instrumentation for intrapericardial pressures, specifically whether an unstressed flat balloon introduced atraumatically is superior under certain circumstances to the familiar catheter techniques. Each yields about the same result with pericardial fluid exceeding 30 ml in experimental animals.

I-6 Therapeutic Myocardial Angiogenesis Using Percutaneous Intrapericardial Drug Delivery
R. J. Laham, M.D., D. Hung, M.D., Ph.D., M. Simons, M.D.

A number of growth factors, including the fibroblast growth factor (FGF) family and vascular endothelial growth factor (VEGF), have all been shown to stimulate blood vessel growth. However, the delivery strategies used were not applicable to patients. The pericardial space appears to play an important role in the physiologic and pathologic regulation of various myocardial processes. Intrapericardial or epicardial administration of various cytokines appear to result in functionally significant angiogenesis in animal models of chronic myocardial ischemia. A single bolus injection of basic FGF (bFGF) into the pericardial space (via a percutaneous subxyphoid access of the pericardial space) improved regional perfusion, regional left ventricular function, and microvascular reactivity, and increased angiographically visible collaterals and magnetic resonance-detected collaterals. The pericardial space, thus, offers an attractive drug delivery reservoir that might be used to deliver therapeutic substances to the heart.

I-10 Pharmacokinetics and Consistency of Pericardial Delivery Directed to Coronary Arteries: Direct Comparison with Endoluminal Delivery

Several proteins were delivered into the porcine pericardial space or into coronary arteries using an endoluminal (EL) delivery catheter, and their penetration into arterial tissue was compared for these two local delivery approaches. The results show that pericardial fluid contents can access coronary arteries with intramural concentrations which vary by 10–15-fold, while EL delivery results in a remarkably wide intramural concentration range with up to 33,000-fold variability. The apparent redistribution rate is more rapid following EL delivery, possibly due to sustained diffusive tissue loading from the pericardial space. Pericardial delivery appears to offer substantial advantages over EL administration with respect to residence time and reproducibility.

I-17 Intrapericardial Treatment of Inflammatory and Neoplastic Pericarditis Guided by Pericardioscopy and Epicardial Biopsy—Results from a Pilot Study
B. Maisch, M.D., S. Pankwept, Ph.D., C. Brilla, M.D., R. C. Funck, M.D., B. C. Simon, M.D., W. Grimm, M.D., M. Herzum, M.D., G. Hufnagel, M.D.

Fourteen patients with autoimmune and 15 with neoplastic effusions underwent pericardioscopy, epicardial and pericardial biopsy with histological, immunohistological and PCR analysis for microbial DNA and RNA. Protusions identified by pericardioscopy proved specif-
ic for neoplastic effusions. Cytology and epicardial but not pericardial biopsy identified neoplastic disorders. Cristalloid triamcinolone (lg intrapericardially) in autoreactive (PCR-negative) pericarditis effectively prevented recurrence in 13 of the 14 cases after 3 months, and in 12 of the 14 cases after one year. In neoplastic effusion 50 mg of intrapericardial cis-platin effectively prevented recurrence of a hemodynamically relevant effusion after 3 and 6 months.

I-23 Efficient in Vivo Catheter-Based Pericardial Gene Transfer Mediated by Adenoviral Vectors

K. L. March, M.D, Ph.D., M. Woody, M.S., K. Mehdi, M.D., D. P. Zipes, M.D., M. Brantly, M.D., B. C. Trapnell, M.D.

It was hypothesized that efficient adenovirus-mediated gene expression in pericardial mesothelium could be achieved by transmyocardial vector delivery to the pericardium. Introduced percutaneously in dogs, the catheter was passed through the right ventricular myocardium. Adenoviral vectors expressing beta-galactosidase, luciferase, or secreted α1-antitrypsin reports were instilled intrapericardially, and reporter gene expression was evaluated. In animals receiving Av1nBg, beta-galactosidase activity was evident in most of the pericardial mesothelium. In animals receiving Av1Lu, luciferase activity was abundant only in the pericardium. In animals receiving Av1Aa, human α1AT was abundant (16–29 µg/ml) in pericardial fluid. The procedure was well tolerated. Thus, highly efficient, minimally invasive adenovirus vector delivery and gene transfer and expression can be induced in the pericardium, supporting the feasibility of intrapericardial gene therapy.

I-30 Initial Clinical Experience with PerDUCER® Device: Promising New Tool in the Diagnosis and Treatment of Pericardial Disease

P. M. Seferovic, M.D., Ph.D., FACC, FESC, A. D. Ristic, M.D., R. Maksimovic, M.D., M.SC., P. Petrovic, M.D., Ph.D., M. Ostojic, M.D., Ph.D., FACC, FESC, S. Simeunovic, M.D., Ph.D., FACC, D. Zamaklar, M.D., D. Simeunovic, M.D., D. H. Spodick, M.D., D.SC., FCCP, FACC

The goal of the present study was to evaluate the feasibility and safety of percutaneous pericardial access with PerDucer® in patients with pericardial disease, and to analyze our initial experience with this new technique. The procedure consists of two distinct techniques: (1) access to the mediastinal space, and (2) pericardial capture, puncture, and insertion of the guidewire. The device was studied in five patients with pericardial disease. Access to the mediastinal space and pericardial capture and probably puncture were accomplished in 4 or 5 patients. However, the authors were not able to confirm the introduction of the intrapericardial guidewire in the pericardial cavity in any of their patients (0/5).

I-36 Minimally Invasive Access of the Normal Pericardium: Initial Clinical Experience with a Novel Device

M. P. Macris, M.D. AND S. R. Igo

Clinical trials are being conducted to evaluate a minimally invasive pericardial access device (PerDUCER”). Twelve clinical trials have been completed on patients undergoing cardiac surgery. In eight patients, a median sternotomy allowed visual positioning of the device prior to pericardial puncture. Four patients underwent a closed-chest, fluoroscopy-assisted procedure. Intrapericardial guidewire insertion was successful in 10 patients (7 on first attempt, 3 on second) without adverse effects. There was no evidence of injury to the pericardium or heart. These studies suggest that the PerDUCER may provide safe, rapid, and effective percutaneous insertion of a guidewire into the normal pericardial space.

I-40 Establishment of a Clinically Correlated Human Pericardial Fluid Bank: Evaluation of Intrapericardial Diagnostic Potential


The development of a clinically correlated human pericardial fluid bank and data base is described. A unique feature of this registry is the availability of a large number of pericardial fluid samples for testing with respect to multiple factors and for correlation with angiographic findings and clinical syndromes expressed by the patients. Study of the pericardial fluid bank should lead to enhanced understanding of molecular mechanisms, as well as the reasons underlying the interpatient variability in these processes. It is further anticipated that this information might provide a foundation for the diagnostic use of pericardial fluid to individualize therapies targeting angiogenesis or plaque physiology.
Introduction

Direct Therapy for Coronary Disease, Myocardial Disease, and Severe Cardiac Arrhythmias

DAVID H. SPODICK, M.D., D.SC., FACC

University of Massachusetts Medical School, Cardiology Division, Saint Vincent Hospital, Worcester, Massachusetts, USA

Delivering treatment directly to the myocardium and the coronary arteries promises a revolution in the management of serious and life-threatening cardiac disease. The pericardial sac provides the route for a variety of treatments aimed directly at the subepicardial structures, essentially everything within the epicardium—that is, the entire heart—utilizing therapeutic agents in concentrations that could not be tolerated systemically, as well as angiogenic factors to increase vascularity in subacute and chronic coronary disease. Participants in this Symposium include investigational and clinical cardiologists, cardiac immunologists, arrhythmologists, and pericardiologists. The work covers investigations in animals demonstrating the effectiveness of agents delivered within the normal pericardial sac for a variety of indications.

The complex physiologic and biochemical characteristics of the normal pericardium also provide the setting for both direct treatment of pericardial disorders and the potential for stimulating this remarkable membrane to produce substances such as prostaglandins with potentially favorable effects on the coronary arteries and myocardium. Access to the pericardium during pericardial effusions (available for many years) would only be incidental, because the vast majority of cardiac patients have normal pericardia with a negligible amount of fluid. To overcome this, an instrument, the PerDUCER®, is undergoing human feasibility and safety trials. Its potential success should mark a new era in cardiac therapeutics.

References


Address for reprints:
D. H. Spodick, M.D.
Cardiology Division
Saint Vincent Hospital
25 Winthrop Street
Worcester, MA 01604, USA
Summary: Intrapericardial delivery of therapeutic agents for pericardial diseases has long been available in the presence of excess pericardial fluid. Most patients with myocardial and coronary disease have no such excess so that their direct treatment requires pericardial access, for which a new instrument has succeeded in animals with induced infarctions, coronary lesions and arrhythmias. Nitric oxide (NO) donors, calcium-avid drugs, antibodies, angiogenic agents (pharmacologic coronary bypass), and hypothermic solutions have been instilled intrapericardially, and even iontophoresis has been used; gene therapy is also promising. Intrinsic pericardiogenic substances may potentially be stimulated for comparable purposes.

Key words: pericardial microphysiology, intrapericardial therapy, coronary disease, myocardial disease, arrhythmias

Intrapericardial Therapy for Pericardial Disease

Nonsurgical intrapericardial therapy has a long history. However, it has been restricted to patients with sufficient fluid in the pericardium (pericardial effusions of sufficient size) for a needle or catheter to be placed safely, and it has been for specific treatment of the causes of such effusions.1 For example, patients with recurrent uremic pericarditis or connective tissue disease, particularly lupus erythematosus, with life-threatening effusions can be treated with corticosteroid agents. Antineoplastic agents have been used for malignancies. With hemopericardium, streptokinase has recently been successful in preventing fibrous organization and adhesions (and subsequent constriction). Similarly, with pyopericardium, streptodornase has been added to streptokinase to destroy the components of pus. Until recently, sclerosants have been used for stubbornly recurrent effusions, particularly in malignancies, but it has been found that leaving a drainage tube in place sufficiently long would adequately sclerose the layers of the pericardium to check further effusion.1

Intrapericardial Therapy for Diseases of the Heart and Coronary Arteries

The prospect of intrapericardial therapy for heart disease targets a rich spectrum of abnormalities, most often with a normal pericardium. Success in animals indicates that appropriate instrumentation is vitally necessary to enter the normal pericardium, which would have only 15–35 ml of fluid, making an extremely thin layer and therefore bringing the pericardium much closer to the heart than with any effusion. It is evident that the direct targeting of the myocardium and the coronary arteries could have advantages in terms of local drug concentration and the prevention or minimization of systemic effects of any therapeutic agent. Animal studies have shown that several agents deposited intrapericardially have a concentration gradient decreasing from the epicardium to the endocardium but that certain antiarrhythmics can penetrate myocardial infarcts.

Experimental Results

Nitric oxide (NO) donors have been used successfully intrapericardially at high concentrations—which, via the venous system, could be detrimental. Willerson et al.2 demonstrated that nitroprusside protects against platelet aggregation in animals with experimental coronary endothelial injury. March utilized NO to significantly decrease coronary wall thickening and lumen narrowing in the porcine over-stretch model of mural response to coronary injury.3

In searching for superior management of dangerous arrhythmias, Zipes and colleagues4 investigated voltage-sensitive chemicals to target depolarized cells in the damaged myocardium and calcium-avid drugs that seek increased intracellular calcium. Antibody techniques were also investigated such that one “head” of an antibody seeks myosin of damaged cells with the other “head” attached to an antiarrhythmic agent. They showed that amiodarone migrates transmurally with significant electrophysiologic effects and appears to suppress induced atrial fibrillation. They also noted that L-arginine decreases the shortening of the tissue effective refractory period and the severity of ventricular arrhythmias (probably due to cardiac NO production).5

Address for reprints:
D. H. Spodick, M.D.
Cardiology Division
St. Vincent Hospital
Worcester, MA 01604, USA
Avitall et al., using the very old technique of iontophoresis via an epicardial patch electrode, achieved high drug concentrations transmurally with penetration of infarcted myocardium. Iontophoresis achieved more rapid suppression of ventricular tachycardia than the usual mechanism for intrapericardial agents—passive diffusion—and also more rapid suppression than intravenous drug.

Uchida et al. achieved a kind of pharmacologic coronary bypass therapy by angiogenic therapy of acute myocardial infarction. Basic fibroblast growth factor (bFGF) given intrapericardially increased the vascularity of the heart in comparison with control animals. There was a greater effect subepicardially (as expected) with angiogenesis and consequent myocardial salvage. In the future, this technique promises treatment for angina as well as infarction.

Dave et al. used the PerDUCER (Comedicus, Inc., Columbia Heights, Minnesota) device to introduce a hypothermic solution during experimental acute myocardial infarction. Hypothermic pericardial perfusion reduced the myocardial temperature and reduced infarct necrosis and infarct size by 50%. (This could also become an adjunct to thrombolysis and to minimally invasive cardiac surgery.)

Landau et al. reported angiogenic therapy of chronic myocardial ischemia, using a rabbit model with A-II induced left ventricular hypertrophy. They gave bFGF intrapericardially and noted marked angiogenesis versus controls, with the effect particularly marked subepicardially. March, with molecular vectors, utilized intrapericardial gene delivery for perivascular and epivascular therapy to achieve transduction of epicardial and parietal pericardial mesothelium. In both swine and dogs, there were no ill effects.

In conclusion, intrapericardially targeted drug therapy and hypothermic superfusion has the advantages of (1) site specificity, (2) superiority to systemic therapy due to increased local concentration and decreased to absent systemic toxicity, and (3) delivery of label-specific therapeutic agents to target cells, receptors, channels, and other structures.

Intrinsic Pericardial Microphysiology:

A Further Target

The rich microphysiology of the pericardium makes it a natural target for investigation of intrapericardial therapy. Microphysiology includes pericardial servomechanisms due to neuroreceptors in the epicardium and the fibrosa, and sympathetic efferents, as well as mechanoreceptors sensitive to changes in ventricular stretch, determined by volume and transmural pressure.

Some of these, along with phrenic afferents, appear to monitor beat-to-beat changes in cardiac volume, while mechanoreceptors with unmyelinated fibers signal myocardial tension and reflexly match contraction strength with peripheral resistance (for example, with exercise). Chemoreceptors sensitive to substances in the pericardial fluid require further exploration.

The pericardial mesothelium has active metabolic activity including metabolism of cyclooxygenase, prostacyclin synthetase, and lipooxygenase. Prostaglandin E2, eicosanoids, and large amounts of prostacyclin (PGI2) are continually released by the mesothelium, especially from the visceral layer into the pericardial cavity. In response to stretch, increased myocardial work and loading, and hypoxia, prostanooids can alter pericardial sympathetic neurotransmission and myocardial contractility and may modulate the caliber and tone of the underlying coronary vessels (i.e., directly via vasodilation by prostaglandin and indirectly by opposing coronary spasm).

A potentially adverse metabolic activity is production of endothelin with a higher pericardial fluid concentration than in all mammalian biologic fluids tested; in congestive heart failure, its concentration is inversely related to the New York Heart Association class. This requires further elucidation, but endothelin is a vasoconstrictor and theoretically may have to be neutralized under certain circumstances.

Conclusion

The experimentally demonstrated and great number of potential avenues for further experimentation in most forms of heart and pericardial disease make the capability to do effective intrapericardial therapy and diagnosis extremely promising. Further investigation, particularly in humans, is urgent.

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Function of the Normal Pericardium

RALPH SHABETAI, M.D.
University of California San Diego; La Jolla CA Veterans Affairs Health Care System, San Diego, California, USA

Summary: Until recently, instrumenting the pericardium was possible only when a pericardial effusion is present or by surgical exposure of the pericardium. Techniques are now being developed to instrument the normal pericardium. This development will allow clinicians and investigators to study pericardial fluid in health and in a variety of disorders not associated with pericardial effusion. It will also be possible to improve our knowledge of pericardial pressure and the pericardial restraints on the heart.

Key words: pericardial physiology, transmural pressure, contact pressure

Background

Until recently, physiologists did not doubt that pericardial and pleural pressures are essentially the same. Thus, both were regarded as intrathoracic pressure, so either one could be used to calculate ventricular transmural pressure, the only difference between the two pressures being the greater deflections relating to the cardiac cycle seen in pericardial pressure tracings. Of particular importance, earlier investigators found that inspiration lowered pericardial pressure almost as much as it did pleural pressure.

We must now consider a seminal paper published many years ago. The investigators infused a liter of saline into the pericardium of dogs. What was observed would not then have been anticipated: the right atrial pressure increased from 3 or 4 millimeters of mercury to 20 millimeters of mercury and its morphology assumed a more dynamic waveform, with large X and Y descents, simulating constrictive pericarditis. A very similar increase in right ventricular diastolic pressure also occurred. The most important finding in the experiment, however, was that pericardial pressure increased almost as much as right atrial pressure and pleural pressure did not change at all. Thus, when one acutely distends the heart by means of a fluid challenge and uses conventional means to measure pericardial pressure with a catheter in the pericardial space, one observes an increase in pericardial pressure, because all biological tissues resist acute strain. The pericardium, however, is stiffer than the myocardium, so when the heart is distended, it engages the pericardium. With further loading of the heart, pericardial pressure rises progressively, so that the change of transmural cardiac pressure is really quite small and much less than the change in absolute, or luminal pressure. Because fluid challenge does not alter pleural pressure when one needs to determine the transmural pressure in ventricular chambers in acute volume overload states, one cannot use intrathoracic pressure but must use pericardial pressure because they would be substantially different. Preload is the transmural pressure, not the absolute pressure. When one wants to study the compliance of the left ventricle, which is critically important in the physiology of diastole, one needs to know the transmural pressure, and to know the transmural pressure one needs to know the pericardial pressure.

Address for reprints:
R. Shabetai, M.D.
Cardiology 111A
La Jolla Veterans Affairs Health Care System
La Jolla, CA 92161, USA
The Concept of Contact Force

Pulmonary physiologists state that a true pleural space does not exist because the two pleural layers are tightly apposed to each other, so that there is only a potential pleural space. It therefore becomes necessary to measure the so-called contact pressure, and that requires a different technique. The technique is to place an unstressed flat balloon in the pleural space and to measure the pressure in the balloon.

Computed tomography of the heart shows minimal pericardial space in normal subjects. It has been proposed that the pericardial space, like the pleural space, is only a potential space and that all classical measurements of pericardial pressure were basically wrong, because liquid pressure, not contact pressure, was measured. Liquid pressure is independent of wherever the catheter tip lies in the space in which pressure is being measured, and consequently is the same wherever the catheter tip happens to lie in the pericardial space. Contact pressure does not share these characteristics.

It has been suggested that the pericardium is like the pleura and that if one introduces a catheter tip into the pericardial space one produces an artifact and would measure a pressure that only exists because the pericardium has been invaded. Contact pressure would be the true constraining force. By analogy, consider, for instance, the contact pressure between the condyle of the femur and the head of the tibia which must be of great magnitude; yet, a needle in the knee joint attached to a transducer would yield only atmospheric pressure. Clearly there are differences between contact pressure and liquid pressure, and the question, of course, is which is relevant to cardiac physiology?

Experiments have been reported in which left ventricular diastolic volume was held constant and left ventricular diastolic pressure was measured before and immediately following pericardiectomy. Pericardial removal caused a substantial drop in left ventricular diastolic pressure, even though the chamber volume was not allowed to change. The difference in left ventricular diastolic pressure before and after pericardiectomy was thus a measure of the pericardial restraining pressure, hereafter termed the theoretical pericardial pressure. This theoretical pericardial pressure was very close to contact pressure measured by a flat balloon placed between the left ventricular wall and the pericardium and was substantially higher than the pericardial liquid pressure measured by conventional catheter technique. The question for the physiologist is which of these different pressures is relevant to normal and abnormal cardiac physiology.

The Influence of Pericardial Effusion

Before the development of techniques to instrument the normal pericardial cavity, the only opportunity for clinical investigators to assess the pressure in a dry pericardial sac was to make the measurement after tapping a pericardial effusion. After pericardiocentesis, however, although the pericardium no longer contains a significant volume of fluid, the pericardium cannot be considered normal, because it was stretched before the tap and it does not recoil after the tap. Pericardial contact pressure would therefore be less than normal.

In animal experiments performed after the pericardium has been aspirated as completely as possible, liquid pericardial pressure becomes markedly subatmospheric, but contact pressure is several millimeters of mercury higher than atmospheric pressure. These low pericardial pressures likely do not exist in normal healthy subjects in whom pericardial fluid is present. When as little as 20 ml of saline is added to the pericardial space, the difference between pressure measured by these different methods disappears. When considering this information, it must be recalled that the human pericardium can normally contain up to 50 milliliters of fluid.

Transmural Cardiac Pressure

Not only is pericardial contact pressure measured from a balloon significantly higher than liquid pressure, it is also virtually the same as right atrial and right ventricular diastolic pressure, which would mean that these chambers operate at a near to zero transmural pressure, defying our concept of preload. How does the idea that contact pressure, not liquid pressure, is the true operating pressure explain why liquid pressure in the pericardium increases during volume overload?

Regional Transmural Pressure

It has long been considered that a film of pericardial fluid serves to equalize gravitational forces around the heart, preventing regional differences in transmural pressures during acceleration and deceleration. This formulation does not apply to contact pressure. Furthermore, when two flat unstressed balloons are placed in different regions of the pericardium, their contact pressures are unequal and change differently during interventions.

Conclusion

The ability to invade the normal pericardium opens opportunities to understand the role of the pericardium in health and in heart disease. Pericardial pressure can now be assessed in euvolemic patients with or without cardiac dilation or hypertrophy, and in acute and chronic overload states. Measurements will be made without altering the preexisting pericardial fluid volume and will be made simultaneously with tiny balloons and by conventional catheter methods.

These advances should improve our understanding of the normal function of the pericardium and its influence when the heart is enlarged, and provide fresh insights into diastolic function of the heart.
Therapeutic Myocardial Angiogenesis Using Percutaneous Intrapericardial Drug Delivery

ROGER J. LAHAM, M.D., DAVID HUNG, M.D., PH.D.*, MICHAEL SIMONS, M.D.

Angiogenesis Research Center, Department of Medicine, Harvard Medical School and Beth Israel Deaconess Medical, Boston, Massachusetts; and the *Cardiovascular Research Division, Chiron Corporation, Emeryville, California, USA

Summary: In this manuscript, we describe the potential role of the pericardial space as a drug delivery reservoir to administer angiogenic agents to the heart resulting in functionally significant angiogenesis with single bolus basic fibroblast growth factor (bFGF) delivery. We also describe a percutaneous subxyphoid pericardial access technique that is safe, rapid, and reliable.

Key words: ischemic heart disease, angiogenesis, fibroblast growth factor, pericardium

Introduction

Ischemic heart disease remains the leading cause of mortality and morbidity in the Western hemisphere. Therapeutic approaches to the management of chronic myocardial ischemia traditionally include efforts aimed at reducing the progression of coronary disease (risk factor modification and aggressive lipid-lowering strategies), reducing myocardial oxygen demand, and preventing cardiac events (medical therapy using antiplatelet agents, beta blockers, angiotensin-converting enzyme inhibitors, and calcium-channel blockers), or increasing blood supply to compromised territories by providing new [coronary artery bypass surgery (CABG)] or restoring old [percutaneous transluminal coronary angioplasty (PTCA)] pathways for blood flow. However, it is becoming clear that a significant proportion of patients with ischemic heart disease are suboptimal candidates for CABG/PTCA and are refractory to medical therapy.1-5 An alternative to these approaches may include an attempt of inducing growth and development of new collateral vessels (therapeutic angiogenesis). Angiogenesis is a complex process involving endothelial cell proliferation and migration, formation of new capillaries, attraction of pericytes and macrophages, stimulation of smooth muscle cell proliferation and migration, breakdown of existing extracellular matrix, formation of new vascular structures, and deposition of new matrix.6-8

The increased expression of various heparin-binding growth factors and their receptors in ischemic myocardium and the ability of these cytokines to induce endothelial cell proliferation and migration in vitro has lead to their rapid development for therapeutic angiogenesis for myocardial and peripheral limb ischemia.6-14

Therapeutic Angiogenesis

We and others have shown that various heparin-binding growth factors including basic fibroblast growth factor (bFGF),15-21 acidic FGF (FGF-1),22 and vascular endothelial growth factor (VEGF)15, 23-25 induce angiogenesis in chronic myocardial ischemia.

Daily injections of 110 µg of bFGF for 18 days directly into the circumflex coronary artery distal to an ameroid occluder hastened restoration of flow in the compromised territory compared with normal saline controls.19 Morphometric analysis of left circumflex (LCx) myocardium demonstrated a significant (2-fold) increase in the number of larger (>20 µm) vessels.19 Daily left atrial injections of 1.74 mg of bFGF for 18 days in the same animal model resulted in early augmentation of coronary flow in the growth factor-treated animals that was comparable with that seen with direct intracoronary injections but was lost by day 38.26 In the same canine model, 7-day systemic arterial administration of bFGF enhanced collateral development without increasing neointimal accumulation at sites of vascular injury.15 Local perivascular delivery of bFGF...
was evaluated in a porcine model of chronic myocardial ischemia (LCX ameroid occlusion). Heparin-alginate microcapsules were used for sustained delivery of bFGF. Animals implanted with heparin-alginate pellets containing 8 µg of bFGF at the time of ameroid placement demonstrated significantly better preservation of perfusion of the ischemic zone during pacing compared with control animals. In addition, ventricular function studies demonstrated better preservation of regional left ventricular function in the ameroid-compromised territory at rest and faster recovery following pacing in bFGF-treated animals.

Examination of the effect of progressively larger amounts of bFGF (10 and 100 µg), delivered in a similar manner in a pig model, demonstrated substantial improvement in resting coronary blood flow in the chronically ischemic myocardium in both bFGF groups compared with controls and an increase in angiographic collaterals. Analysis of left ventricular function demonstrated a higher ejection fraction at rest and during pacing in both 10 and 100 µg bFGF groups compared with controls.

Treatment with VEGF also results in a myocardial angiogenic response in animal myocardial ischemia models. A study carried out in a dog ameroid model suggested that daily intracoronary injections of 45 µg of VEGF delivered distal to the occluder over a 28 day period (total dose 900 µg) resulted in faster restoration of collateral zone flow than did similar injections of normal saline. Morphologic analysis demonstrated a significantly higher number of small vessels in VEGF-treated compared with control animals. The therapeutic efficacy of VEGF in porcine circulation was tested using an implantable minipump primed with 2 µg of VEGF and 50 U of heparin delivered over 4 weeks periadventitially to the circumflex coronary artery distal to the ameroid occluder. Comparison of VEGF/heparin- and heparin only-treated animals demonstrated that while coronary flow in the ischemic territory at rest was no different between the two groups, VEGF treatment was associated with better coronary flow during pacing. Assessment of myocardial perfusion using magnetic resonance imaging demonstrated not only significantly better perfusion of the compromised territory in VEGF-treated animals but also a reduction in the size of this territory. Morphometric analysis found a nearly 4-fold increase in the number of collateral vessels in VEGF-treated animals compared with control animals. Analysis of left ventricular function demonstrated a higher ejection fraction at rest and during pacing in both 10 and 100 µg bFGF groups compared with controls.

The efficacy of VEGF (20 µg) single bolus intracoronary injection was compared with the same amount of VEGF delivered either perivascularly or locally using an InfusaSleeve catheter (LocalMed, Inc., Palo Alto, Calif., USA). The studies conducted in a porcine ameroid constrictor model demonstrated that, compared with control animals, both intracoronary bolus injection and local delivery resulted in significant increase in angiographically detected left-to-left collaterals and improvement in myocardial blood flow, regional left ventricular function, and microvascular function.

The role of the pericardium

The pericardium likely plays an important role in the regulation of several myocardial processes. The concentration of bFGF and VEGF in the pericardial fluid of patients with unstable angina is significantly higher than in patients with nonischemic heart disease. The concentration of bFGF in pericardial fluids in ischemic patients was 2036 ± 357 pg/ml, significantly (p < 0.001) higher than 289 ± 72 pg/ml in nonischemic patients. The concentration of VEGF in the pericardial fluid tended to be higher in ischemic patients, but the difference was not statistically significant (39 ± 7 vs. 22 ± 6 pg/ml).

The increased levels of such endogenous proangiogenic factors in the pericardial fluid of patients with ischemic heart disease suggest a physiologic role for these factors in the host response to myocardial ischemia and injury. This observation coupled with the favorable pharmacokinetic profile of some drugs in the pericardial space suggests that the pericardial space may potentially serve as a unique drug delivery reservoir for the delivery of therapeutic agents to the heart.

Therapeutic Angiogenesis Using Pericardial Delivery

Landau et al. studied the effects of an intrapericardial bFGF infusion in a model of chronic myocardial ischemia. Intra-venous angiotensin II (AII) was infused to induce left ventricular hypertrophy and concomitant ischemia in New Zealand white rabbits. Basic fibroblast growth factor was infused into the intrapericardial space using an osmotic pump. Epicardial angiogenesis was graded histologically on a scale of 0 to 2. Animals receiving intravenous AII displayed left ventricular hypertrophy. A highly localized angiogenic effect of bFGF was observed compared with control animals. Uchida et al. studied the effect of intrapericardial bFGF (30 µg bFGF + 3 mg heparin) in a canine model of acute myocardial infarction. One month later infarcted weight/left ventricle weight was 24 ± 5.2%, 25 ± 4.0, 18 ± 2.4, and 10 ± 1.8% with saline, heparin, bFGF alone, and bFGF + heparin administration, respectively. Vascular number in the infarcted area of the outer layer was the largest in the bFGF + heparin group (13 ± 3.3, 20 ± 2.2, 47 ± 8.3, and 136 ± 26.3 in the saline, heparin, bFGF alone, and bFGF + heparin groups, respectively). The vascular
number was larger in the subepicardial than in the subendocardial infarcted areas.20

Thus, intrapericardial administration of growth factors results in histologic evidence of neovascularization18,20 and a reduction in myocardial infarction extent.20 The infusion of growth factors in these studies and the hypertrophy model and acute myocardial infarction model in these studies limits their applicability to patients with ischemic heart disease secondary to progressive atherosclerosis. Therefore, a single dose intrapericardial administration of angiogenic growth factors in a chronic myocardial ischemia model was needed utilizing the pericardium as a drug delivery reservoir to deposit these therapeutic agents.

Therapeutic Angiogenesis Using Single-Dose Intrapericardial Administration of bFGF

We have recently completed an animal study of intrapericardial bFGF delivery for therapeutic angiogenesis in a porcine chronic myocardial ischemia model, using a percutaneous subxyphoid pericardial access technique.32–34 Forty-nine Yorkshire pigs underwent ameroid placement on the LCx artery. Three weeks after ameroid placement, animals underwent coronary angiography to confirm LCx occlusion and to assess the extent of angiographic collaterals. After microsphere injection and determination of regional left ventricular function, myocardial perfusion, and collateral extent using magnetic resonance imaging,24, 35 the animals underwent percutaneous (nonsurgical) subxyphoid access of the pericardial space using a blunt-tipped needle under fluoroscopic guidance.32 Access of the pericardial space was confirmed by the injection of 1 ml of diluted contrast.32 Then, the animals were randomized to receive bFGF (30 µg, 200 µg, or 2 mg) or saline/heparin. Four weeks later, the animals underwent repeat angiography, left atrial microsphere injection, magnetic resonance imaging for regional left ventricular function, myocardial perfusion, and collateral extent. They were then sacrificed and the hearts were excised for morphometric analysis of microsphere blood flow determination, and measurement of microvascular function to determine endothelial-dependent and -independent vasodilation.28, 33, 36–38

Percutaneous subxyphoid access of the pericardium was successful in all animals without any complications,32 and drug delivery was accomplished in all cases. Three weeks after implantation of ameroid occluders, at the time of intrapericardial drug delivery, myocardial vascular resistance in the collateral-dependent LCx territory was similar in all treatment groups and was significantly higher than resistance in the left anterior descending (LAD) territory. Four weeks following intrapericardial drug delivery, LCx resistance was significantly lower in bFGF-treated animals than in controls,33 and bFGF treatment resulted in a significant increase in angiographic collaterals, regional myocardial blood flow, myocardial function in the ischemic territory, collateral extent, and myocardial vascularity compared with control animals.34 Microvascular analysis showed that endothelium-dependent vasodilation was normal in the LAD but not in the LCx distri-

bution in control animals, indicating dysfunctional endothelium in the ischemic zone; however, bFGF treatment improved endothelium-dependent vasodilation in the LCx epicardium.33 Thus a single bolus administration of bFGF in a porcine model of chronic myocardial ischemia resulted in functionally significant angiogenesis.

Conclusion

The pericardial space appears to play an important role in the physiologic and pathologic regulation of various myocardial processes. The elevated levels of various angiogenic cytokines in patients with myocardial ischemia indicate a potential role of the pericardium in ischemia-induced angiogenesis. Intrapericardial or epicardial administration of these cytokines appear to result in functionally significant angiogenesis in animal models of chronic myocardial ischemia. The above-described study demonstrated the ability of a single bolus injection of bFGF into the pericardial space to induce myocardial angiogenesis and improve regional perfusion, regional left ventricular function, and microvascular reactivity, and to increase angiographically visible collaterals and magnetic resonance-detected collaterals. Thus, the pericardial space offers an attractive drug delivery reservoir that might be used to deliver therapeutic substances to the heart.

References


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Pharmacokinetics and Consistency of Pericardial Delivery Directed to Coronary Arteries: Direct Comparison with Endoluminal Delivery

HANS-PETER STOLL, M.D.,* KATHY CARLSON, B.A.,† LARRY K. KEEFER, PH.D.,‡ JOSEPH A. HRABIE, PH.D.,§ KEITH L. MARCH, M.D., PH.D. FACC*||

*Krannert Institute of Cardiology and †Department of Radiology, Indiana University School of Medicine, Indianapolis, Indiana; ‡Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center; §Chemical Synthesis and Analysis Laboratory, SAIC Frederick, NCI-FCRDC, Frederick, Maryland; ||Richard L. Roudebush Veterans Administration Medical Center, Indianapolis, Indiana, USA

Summary

Background and hypothesis: Pharmacologic modulation of the contents of the pericardial space has been shown to influence the response of coronary arteries to balloon injury. Endoluminal (EL) local delivery of various drugs into coronaries has been found to be limited by short residence time, as well as by highly variable deposited agent concentration. We hypothesized that compounds placed into the pericardial space (P) would penetrate into coronary tissue with greater consistency than seen after EL delivery and provide for prolonged coronary exposure to agents.

Methods and Results: 125I-labeled basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), albumin, or 131I-labeled diazeniumdiolated albumin (NONO-albumin) were delivered as model/therapeutic proteins into the porcine pericardial space (n = 15 pigs) or into coronaries using an EL delivery catheter (n = 48 arteries). In subjects receiving 125I-labeled proteins, the delivery target or mid-regions of the left anterior descending (LAD) and left circumflex (LCx) arteries were harvested at 1 h or 24 h for gamma-counting and autoradiography, and fractional intramural delivery (FID) or retention measured as percent agent in 100 mg artery/agent in infusate for both time points. In the animals receiving 131I-labeled NONO-albumin, serial gamma imaging was employed to evaluate the rate of redistribution in individual animals following either pericardial or endoluminal delivery. At 1 h, FID values ranged from 0.00064 to 0.0052% for P delivery (median 0.0022%), and from 0.00021 to 6.7 for EL delivery. The estimated T1/2 for bFGF redistribution from the vascular tissue was 22 h (P) and 7 h (EL), respectively, while the directly determined T1/2 values for NONO-albumin redistribution from the delivery region were 22.2 h (P) and 2.5 h (EL).

Conclusions: These data show that pericardial fluid contents can access coronary arteries with intramural concentrations which typically vary by 10–15-fold, while EL delivery results in a remarkably wide intramural concentration range with up to 33,000-fold variability. The apparent redistribution rate is more rapid following EL delivery, possibly due to sustained diffusive tissue loading from the pericardial space. Pericardial delivery appears to offer substantial advantages over EL administration with respect to residence time and reproducibility.

Key words: pericardium, coronary disease, local drug delivery, restenosis, angiogenesis
to achieve deposition of therapeutic agents\(^2\) has demonstrated substantial equivalence among these devices in the pattern and level of drug deposition achieved.\(^6\) However, endoluminal deliveries by these are routinely characterized by several limitations: (1) inconsistency in delivery, (2) low absolute efficiency of localization, and (3) relatively rapid washout of agent from the target vessel.\(^7\)

The recent advent of catheter-based approaches to pericardial access has made possible the evaluation of agent delivery directly into this space with the eventual goal of clinically useful therapies. We hypothesized that drug delivery into the pericardial sac would differ from endoluminal deliveries by (1) comparatively enhanced consistency, and (2) prolonged exposure of either coronary or myocardial tissues to drug as a result of a reservoir function of the pericardium.

This study was designed to provide a direct comparison of endoluminal with intrapericardial delivery for selected agents which could be evaluated following delivery by these approaches. For endoluminal deliveries we employed a microporous infusion catheter (MIC; Cordis Corp., Miami, Fla., USA) consisting of a flow-restricting inner balloon with multiple 25 \(\mu\)m holes and an outer balloon membrane with 0.8 \(\mu\)m pores, which provides a “weeping” convective transport of the infused drug during balloon inflation.\(^2\) For intrapericardial deliveries, we employed either a hollow, helical-tipped catheter designed for controlled penetration through the myocardium during fluoroscopic visualization,\(^8\) or a sheathed needle with a suction tip designed for grasping the pericardium and accessing the pericardial space using a transthoracic approach while avoiding myocardial puncture.

The substances chosen for this study were proteins with an anticipated potential for therapeutic effect following local delivery, as well as additional model proteins. These included (1) albumin diazeniumdiolate (NONO-albumin), a potentially anti-restenotic agent; (2) basic fibroblast growth factor (bFGF), an agent currently under evaluation for its proangiogenic effects; and (3) albumin and (4) platelet-derived growth factor (PDGF)-BB, two model proteins with minimal and substantial receptor-binding affinities, respectively. These were delivered either using the endoluminal catheter, or by a pericardial delivery catheter as described below, and the consistency as well as rate of redistribution of delivered substance were noted.

Materials and Methods

Study Design

This study involved four experimental groups: (1) Endoluminal delivery of \(^{125}\)I-labeled bFGF, PDGF, or albumin followed by timed quantitation of agent placed; (2) pericardial delivery of \(^{125}\)I-labeled bFGF followed by timed quantitation; (3) endoluminal delivery of \(^{131}\)I-labeled NONO-albumin followed by serial gamma imaging of the cardiac region; and (4) pericardial delivery of \(^{131}\)I-labeled NONO-albumin also followed by serial gamma imaging. Two arteries were subjected to local delivery in each of 24 animals receiving endoluminal deliveries for acute (\(n = 24\) arteries) or 24-h (\(n = 24\) arteries) evaluation (Group 1); two arteries were also harvested and studied in each of 12 animals receiving pericardial delivery for acute (\(n = 12\) arteries) or 24-h (\(n = 12\) arteries) evaluation (Group 2); 1 animal was imaged serially after a single endoluminal delivery (Group 3); and 3 animals were imaged following pericardial delivery (Group 4). All experiments and animal care conformed to National Institutes of Health and American Heart Association guidelines for the care and use of animals, and were approved by the Animal Care and Use Committee of Indiana University.

Endoluminal Delivery Procedure

In all, 25 domestic swine (20–23 kg in weight) were premedicated with 375 mg oral aspirin on the day prior to the study. Animals were sedated with a combination of ketamine (20 mg/kg), acepromazine (1.1 mg), and atropine (0.6 mg/kg) by intramuscular injection. The animals were given pentobarbital sodium (300 mg) intravenously and were then intubated. They were ventilated mechanically with isoflurane 2% and oxygen 90% (\(2 \text{L/min}\)) using a respirator. The electrocardiogram (ECG) and intra-arterial blood pressure were monitored continuously throughout the procedure. Arterial access was made via surgical cutdown of a carotid artery or femoral artery, and an 8F introducer sheath was placed. Each animal received a single dose of heparin (5000 U) and bretylium tosylate (2.5 mg/kg). Under fluoroscopic guidance, an 8F guiding catheter was positioned in the left coronary ostium. After the intracoronary administration of nitroglycerin (100 \(\mu\)g), coronary angiography was performed in the anterior-posterior or 30° left anterior oblique (LAO) position. After review of the angiogram, a 3.0 mm diameter segment of each of two coronary arteries [left anterior descending (LAD) and left circumflex (LCx)] was selected so as to minimize side branches or tortuosity. In each case, measurements were performed using spot AP films and digital calipers. Coronary overstretch balloon injury at each selected site was performed by angioplasty twice for 15 s, each time using a noncompliant angioplasty balloon, 0.5 mm in diameter larger than the luminal diameter. Following balloon injury, a delivery balloon was selected to match the nominal diameter of the balloon used for the initial injury in each case, prepared as per the manufacturer’s directions, and placed at the midpoint of the injured segment. The agent to be delivered was infused in a volume of 2 ml, at a pressure of 3 atmospheres as measured at the operator end. The second target vessel then received percutaneous transluminal coronary angioplasty (PTCA) and local delivery in a similar fashion using a new catheter. These procedures were performed in 49 arteries (8 coronary arteries/agent \(\times 2\) time points in Group 1, and 1 LAD delivery in Group 3).

Pericardial Delivery Approaches

Pericardial deliveries were performed by either a percutaneous transventricular method, or a transthoracic approach. The transventricular method employed a hollow, helical-tip-
ped catheter designed for controlled penetration through the myocardium into the pericardial space during fluoroscopic visualization. Following placement of a 7F sheath into the right carotid artery, a catheter was placed through the sheath and advanced under fluoroscopic guidance into the left ventricle to the cardiac apex, with the catheter tip directed inferiorly. Upon firm contact with the myocardium, the catheter tip was advanced through the myocardium using a gentle turning motion. After advancement over several mm, hand infusion of a 1:1 meglumine/normal saline mixture was initiated and contrast location monitored fluoroscopically. Successful intrapericardial tip placement was identified by accumulation of contrast in the pericardium, at which point the catheter was fixed in position and flushed with 1 ml of saline prior to delivery of the desired agent in a volume of 10 ml. Following delivery, final catheter position was confirmed by fluoroscopic visualization of a bolus of air instilled into the pericardial space, after which the catheter was removed.

The transthoracic approach used in a subgroup of pericardial deliveries involved a sheathed needle with a suction tip designed for grasping the pericardium and accessing the pericardial space using a transthoracic approach while avoiding myocardial puncture. This device was placed from a subxiphoid position into the mediastinum under fluoroscopic guidance and positioned onto the anterior outer surface of the pericardial sac. The sac was then retracted under manual suction, entered by the needle, and a guidewire was placed through the needle lumen into the pericardial space. The wire was advanced several cm in order to identify a configuration which reflected intrapericardial position, after which the needle was removed and a 4F dilator catheter (Cook Inc., Bloomington, Ind.) inserted over the wire. Following removal of the wire, successful intrapericardial tip placement was confirmed by accumulation of infused contrast in the pericardium, at which point the desired agent was delivered in a volume of 10 ml and the catheter was finally removed.

Agents for Delivery

For endoluminal delivery, $^{125}$I-labeled bFGF, PDGF-BB, and albumin were obtained from Dupont/NEN (Wilmington, De.) and each was dissolved in a solution of bovine serum albumin (BSA) in order to minimize nonspecific binding to the catheter lumina. For pericardial delivery, $^{125}$I-labeled bFGF was dissolved in an excess of 200 µg cold bFGF either with or without the addition of 3 mg heparin. Bovine serum albumin was conjugated with nitric oxide-releasing diazeniumdiolate groups as described elsewhere (Hrabie et al., personal communication) and was labeled with $^{131}$I by Covance Laboratories, Inc. (Vienna, Va.), using the chloramine-T method; the sample was purified to radiochemical homogeneity by Sephadex chromatography and shipped to the Krannert Institute at a specific activity of 99 mCi/mg. This conjugated BSA (NONO-albumin) possessed approximately 30 moles NO/mole albumin and was used for both endoluminal and pericardial delivery. For gamma imaging studies, a dose of 20–25 µCi was coadministered either via the endoluminal or pericardial approaches, admixed with 40 mg of cold BSA-diazeniumdiolate dissolved in saline at a pH of 7.4.

Imaging and Computer Processing for Evaluation of Regional Nature of Delivery

A Pho-gamma LFOV scintillation camera 6413 with a medium-energy (300 keV) collimator (Searle Radiographics, Des Plaines, Ill.) that allowed visualization of the thoracic region of interest was used for imaging. The images were acquired into an ADAC 33000 computer using a $128 \times 128 \times 8$ matrix. A known amount (about 10 µCi) of the $^{110}$I-labeled compound was put in a $13 \times 100$ mm tube, placed in a styrofoam at a distance from the $\gamma$-camera that equalled the distance between the camera and the heart, and positioned adjacent to the animal during imaging. It acted as a control throughout the experiment. Background radiation was checked and recorded. Acquisition of serial 10 min anteroposterior planar images began immediately following infusion. After each static image, the pig was repositioned for a lateral 10 min image of the thorax. On each image, two or three areas of interest were drawn for separate quantitation: one around the external reference source, one restricted to the activity in the cardiac region, and one restricted to the most intense area of activity discerned in the mid-region of the heart, in the animal receiving endoluminal delivery into the mid-LAD. Counts were recorded from each area of interest and were corrected for decay.

The count rate was assessed, and the radioactivity/pixel was viewed in a matrix format with a pixel size of 0.19 mm/pixel. The total activity in each region of interest after correction for background and decay was plotted as a function of time to yield a local half-time of agent loss.

Preparation of Samples Containing $^{125}$I-Labeled Agents and Evaluation of Delivery Efficiency

Arteries infused by the endoluminal catheter (n = 48) with each $^{125}$I-labeled agent above, as well as those exposed to intrapericardial $^{125}$I-labeled bFGF with or without admixed heparin (n = 24) were obtained unfixed at either 1 h or 24 h following either delivery. The coronary artery segment region subjected to the endoluminal delivery (approximately 25–30 mm in length) was isolated from the underlying musculature or connective tissue, as were homologous segments in the case of intrapericardial delivery. These arterial samples were then subjected to well-counting for the amount of contained radioactivity. An aliquot of the infusion solution was measured as a control in each case. Results were corrected for background radioactivity, amount of injected radioactivity, and tissue weight. These results are expressed in terms of a fractional intramu-
Results

Feasibility of Catheter-Based Intrapericardial Delivery

In this study, a total of 15 animals received direct intrapericardial delivery using catheter techniques not requiring an open surgical approach. Entry into the pericardial sac was typically accomplished in less than 5 min following initial access, and no acute or subacute (24 h) complications of pericardial access were noted. Specifically, there was no evidence of progressive increase in intrapericardial fluid volume following the delivery, pericardial tamponade, or remarkable inflammation upon tissue harvest in any animal.

Reproducibility of Endoluminal versus Intrapericardial Delivery

The FID obtained from a determination of the activity present in each isolated target vessel acutely following delivery is shown in Figure 1A for both endoluminal and pericardial deliveries. The endoluminal approach is seen to be characterized by an extreme variability in FID, with an observed overall minimum of 0.0002% and maximum of 6.7%, resulting in a 33,000-fold range of variation, despite all experiments being conducted as consistently as possible with respect to catheter type, handling, sizing, infusion pressure, and tissue analysis. Wide variability was seen to be present for each of the three proteins infused. The pericardial instillation of bFGF showed a remarkably different profile, with a 10-fold range of FID. This comparatively tight clustering of data was present both for the bFGF/heparin and bFGF groups (the latter displayed less than 3-fold variability).

The FIR determined for each vessel 24 h following either endoluminal or pericardial delivery is shown in Figure 1B. The endoluminal approach is again characterized by remarkable variability, with the FIR displaying a 6,000-fold dynamic range again, despite every effort to optimize delivery consistency. Once again, the variability is present for each of the three proteins tested. The pericardial instillation of bFGF showed a persistent clustering of activity present both for the bFGF/heparin and bFGF groups, with a 30-fold range of FIR. Although computation of local pharmacokinetics and residence half-times is rather limited using such data sets that are obtained from measurements of unrelated samples with large in-group variability, an estimation may be made of the redistribution T_{1/2} of bFGF for the endoluminal and pericardial groups using the median values for FID (1 h) and FIR (24 h). This approach yields a value of 7 h for the endoluminal group and about 24 h for the pericardial group.

Geometry and Pharmacokinetics of Endoluminal versus Intrapericardial Delivery: NONO-Albumin

Each of three animals receiving intrapericardial delivery of 131I-labeled NONO-albumin was imaged acutely following the instillation, as well as at multiple time points subsequently. A representative pair of planar gamma images is depicted in Figure 2, showing both an anterior (A) and left lateral view (B) of the cardiac region obtained immediately after delivery. Such planar images reflect a sum of intrapericardial, intramyocardial, and blood pools of radioactive material. The activity is seen to be highly circumscribed, as expected for an agent predominantly localized to the region within the pericardium following initial deposition. This pattern was persistent during the subsequent imaging in each animal. A degree of heterogeneity of the activity/pixel was present, as is apparent in the pseudocolor image rendering, in which the intensity ranges from dark red (least activity) to bright yellow (most activity). The activity distribution tended to suggest a higher collection of agent laterally and inferoapically, consistent
with the intrapericardial distribution of radiographic contrast typically found to predominate after delivery of volumes in the order of 10 ml in the supine position. This distribution of liquid in the nondiseased pericardium presumably reflects the dynamic interaction of anatomic attachments, gravity as applied in the supine position, and myocardial contractile activity resulting in continuous mixing.

A typical sequence of images observed over a time course following endoluminal delivery of 131I-labeled NONO-albumin is shown in Figure 3A. The animal in this study received an infusion of 2 ml of solution into the mid-LAD, with the resultant image revealing a tightly restricted regional delivery of intensity surrounding this area. It is apparent from the series that the NONO-albumin, which possesses a molecular weight of about 72 kDa, is redistributed from the arterial region to a very significant degree within the first several hours after infusion. By contrast, the sequence of images observed following pericardial delivery of the same agent (Fig. 3B) shows a markedly prolonged redistribution time from this compartment. Furthermore, each of these images shows a larger projected area of activity associated with pericardial rather than periarterial localization.

A determination of fractional regional delivery (delivery efficiency) for each form of delivery could be obtained as a ratio of the activity detected in the region of interest immediately after delivery to the total activity infused. In the pericardial deliveries, this value was not appreciably different from 1.0 (100%), while for endoluminal delivery the initial activity was 8.7% of that infused.

Fitting of the quantitative data derived from these serial acquisitions demonstrated that the redistribution of NONO-albumin could be fit closely by a monoexponential decay function. This was true for both the endoluminal and the pericardial delivery conditions. Figure 4 shows the decrease in radioactivity within the regions of interest as a function of time for the pericardial deliveries averaged (A), and for the endoluminal delivery (B). The data displayed following the endoluminal delivery reflect the overall cardiac region (identical in
size/shape to that in the pericardial deliveries), as well as a local region of interest drawn immediately surrounding the site of maximal intensity in the mid-LAD region. The rate of decrease of agent over the local site of endoluminal delivery was best fit by an exponential function with a $T_{1/2}$ of 2.5 h. Similar fitting of the data acquired over the entire cardiac region of interest yielded a function with $T_{1/2}$ of 3.9 h. However, following intrapericardial delivery, the agent was washed out with an average $T_{1/2}$ of 22.2 h (range 14.3–27.3 h), or ninefold more slowly than following the endoluminal delivery.

**Discussion**

The prospect of using local delivery of bioactive agents to target their effect to a specific locus of disease in the context of the cardiovascular system has attracted much interest over the past several years, but only a few studies to date have validated the efficacy of particular locally applied drugs in large animal models of human vascular pathology. Several aspects of local drug delivery targeting the arterial wall have been identified as potential reasons for the difficulty in demonstrating such efficacy: low efficiency of localization, rapid agent washout in the absence of specific measures to prolong residence time, and poor reproducibility of local delivery.

The development of delivery devices which are practical for nonsurgical clinical access to the pericardial space has set the stage for the evaluation of pericardial delivery as a possible approach to address these challenges. However, there have been few studies directed to careful characterization of the features of pericardial delivery, particularly as contrasted with the more traditional forms of endoluminal delivery. Moreover, there has been little analysis of the ability of material infused into the pericardial space to access the tissues of the coronary arteries. This study was thus designed to provide direct insight into the comparative features of pericardial and endoluminal delivery, specifically with respect to delivery consistency and pharmacokinetics.

The most striking finding of this study is the extreme variability found in the quantitated amount of material deposited locally following endoluminal delivery using a highly standardized approach. This is not entirely unanticipated, considering the results of an earlier multicenter animal study which also demonstrated substantial variability in the intensity of deposition of a fluoresceinated oligonucleotide antisense to the c-myc oncogene. However, this study was only semiquantitative in nature, so that the degree of variability was not apparent.

The explanation for such generalized lack of reproducibility in the cardiovascular system has attracted much interest over the past several years, but only a few studies to date have validated the efficacy of particular locally applied drugs in large animal models of human vascular pathology. Several aspects of local drug delivery targeting the arterial wall have been identified as potential reasons for the difficulty in demonstrating such efficacy: low efficiency of localization, rapid agent washout in the absence of specific measures to prolong residence time, and poor reproducibility of local delivery.

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data clearly demonstrate that the local rate of washout of NONO-albumin is significantly more rapid ($T_{1/2}$ of 2.5 h) following endoluminal delivery into the LAD than after delivery into the pericardial space ($T_{1/2}$ of 22 h). This may be so due to the fact that the redistribution rate would be expected to depend directly on the local blood flow, so that the pericardial contents themselves would be rapidly washed out only after access to the myocardial tissues, while endoluminally delivered agents would be in a comparatively high-flow environment from the time of their initial deposition.

Conclusions

This study continues a series of reports focusing on intrapericardial delivery and adds to a growing literature establishing its feasibility using catheter-based, nonsurgical methods.\textsuperscript{21–24} The data presented here indicate that pericardial fluid contents can access coronary arteries with intramural concentrations which vary by 10–15-fold, while EL delivery results in an remarkably wide intramural concentration range with up to 33,000-fold variability. Also, the apparent redistribution rate is substantially more rapid following EL delivery, possibly due to sustained diffusive tissue loading from the pericardial space. Pericardial delivery thus appears to offer substantial advantages over EL administration with respect to both reproducibility and residence time.

As such, it is anticipated that clinical trials employing pericardially delivered agents directed to angiogenesis, restenosis, and perhaps other coronary and myocardial indications will emerge over the next several years.

Acknowledgments

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References

Intrapericardial Treatment of Inflammatory and Neoplastic Pericarditis Guided by Pericardioscopy and Epicardial Biopsy—Results from a Pilot Study

BERNHARD MAISCH, M.D.,* SABINE PANKUWEIT, PH.D.,† CHRISTIAN BRILLA, M.D.,‡ REINHARD C. FUNCK, M.D., BERND C. SIMON, M.D., WOLFRAM GRIMM, M.D.,‡ MATTHIAS HERZUM, M.D., GÜNTER HUFNAGEL, M.D.

Department of Internal Medicine and Cardiology, Philipps-University, Marburg, Germany

Summary: From a registry of 136 patients undergoing pericardiocentesis, 14 patients with autoimmune and 15 patients with neoplastic effusions were selected. All underwent pericardioscopy, epicardial and pericardial biopsy with histologic, immunohistologic, and polymerase chain reaction/or in situ hybridization analysis for microbial DNAs and RNA. Pericardioscopy identified neoplastic effusions by the high occurrence of protrusions. Fibrin threads and layers and neovascularization were found in both groups. For identification of the inflammatory and neoplastic process, the combined analysis of the cytology of the effusion and epicardial biopsy evaluation proved to be most important. Epicardial biopsy demonstrated a slightly higher sensitivity for identifying neoplastic disorders in the pericardium than cytology alone. Pericardial biopsy was inconclusive. Intrapericardial administration of 1 g of crystallloid triamcinolone in autoreactive pericarditis prevented recurrence in 13 of the 14 cases after 3 months and in 12 of the 14 cases after 1 year. In neoplastic effusion, intrapericardial administration of 50 mg cis-platin for 24 h prevented recurrence of a hemodynamically relevant effusion after 3 months in all, and after 6–12 months in 14 of 15 patients. Mortality in neoplastic effusion due to noncardiac tumor progression was 47 and 80%, respectively, after 3 and 6 months, as can be expected in endstage neoplastic disease. This pilot study demonstrates that local drug application is feasible, lifesaving, and well tolerated by the patients. It opens perspectives for local drug application in other cardiac disorders as well.

Key words: pericardioscopy, pericardial effusion, epicardial biopsy, neoplastic pericardial effusion, triamcinolone, cis-platin

Introduction

The causes of pericardial effusion are manyfold. Viral, bacterial, fungal or rickettsial infections, radiation, renal failure, severe heart failure, hypertrophic and dilated cardiomyopathy, or storage diseases may induce it. It is observed after cardiac surgery, after trauma or transmural infarction, after post-transfusion, and in neoplastic disorders (reviewed in Refs. No. 2 and 23).

Echocardiography, computed tomography, and magnetic resonance imaging are all used by clinicians to assess the presence and size of pericardial effusion. Macroscopic evaluation was restricted to the pathologist or the surgeon until transcutaneous pericardioscopy and epicardial and pericardial biopsies revealed macroscopic and microscopic evidence of inflammatory or neoplastic pericardial and epicardial injury. These methods add positively to the analysis routine assessment of the pericardial fluid for enzymes, density, and hemoglobin, and cells by cytochemistry. It facilitates epicardial and pericardial biopsies both of which are fairly new investigative techniques. Pericardioscopy and epicardial biopsies are obviously the prerequisite for polymerase chain reaction (PCR) assessment of microbiologic etiology.

For the treatment of so-called idiopathic pericardial effusion, nonsteroidal antiphlogistics, colchicine, and prednisone or prednisolone have been used widely, sometimes in controlled, rarely in randomized, and never in double-blind, randomized multicenter trials (reviewed in Ref. No. 2). For this reason, we examined in this pilot project the acute and long-term effect of 1 g triamcinolone acetate in crystallloid form given intrapericardially for 24 h. In neoplastic pericardial dis-
malignant pericardial effusion may cause fatal tamponade or cardiac failure. Diagnostic measures may identify the underlying malignant growth when analyzed directly in the pericardial fluid or in the biopsies.

Whereas tamponade can be relieved by pericardiocentesis, surgical or transcutaneous pericardiotomy is the treatment course for preventing recurrence in addition to systemic application of antineoplastic drugs. Therefore, this report also focuses on the effect of an intrapericardial application with cis-platin in patients with large effusion or tamponade from malignant pericardial effusions.

**Patients and Methods**

**Patients**

This report details our experience in 29 patients undergoing pericardioscopy and epicardial and pericardial biopsy. In 14 patients with autoimmune pericardial effusion, 1 g/24 h triamcinolone acetate was administered intrapericardially. In 15 patients, 50 mg/cis-platin was given intrapericardially. All patients were selected from our registry of 136 patients who had undergone pericardiocentesis puncture from 1989 to 1998 in our department. The patient cohort with autoreactive pericardial effusion (n = 14) comprised 5 male and 9 female patients, with an age range from 7 to 17 years. The neoplastic treatment group comprised 15 patients, 9 male, 6 female, with an age range from 47 to 78 years. In all patients, echocardiography was carried out from the parasternal, apical, and subxyphoid window. A prerequisite for both the puncture and the procedure was the echofree zone of > 4 to 5 mm in diastole in the subxyphoid view. Cardiac tamponade was present in 8 of 14 patients with autoimmune pericarditis and in 13 of 15 patients with neoplastic disorders.

**Methods and Pericardioscopy Procedure**

The diagnostic approach to the diagnosis of pericardial effusions is demonstrated in Figure 1.

Pericardiocentesis and pericardioscopy with optically guided biopsies from the epicardium and pericardium can be carried out as an emergency or elective measure, depending on the hemodynamic compromise derived from the effusion. Pericardiocentesis and pericardioscopy were carried out after local anesthesia in the subxyphoid region. Then a 1.4 mm cannula was advanced under radiographic control until pericardial fluid was aspirated. A teflon-coated exchange wire (Cordis) was then introduced, and the cannula was removed and exchanged for a 9F introducer set (Cordis). The length of the outer sheath was adapted to the length of the rigid pericardioscope minus 0.5 cm. A pigtail catheter was introduced and the pericardial fluid was removed gently by an electric suction syringe. Samples of the fluid were preserved for cytology, immunology, immunocytochemistry, bacteriology, virology, pathology, and the determination of standard laboratory parameters such as leukocyte count, Hb, HbE, protein content, and enzymes [lactose dehydrogenase (LDH), creatine kinase (CK), CK-MB, amylase]. Then 100–150 ml volumes of saline (37°C) were repeatedly injected into the pericardium and removed until the fluid from the pericardial sac was clear and permitted pericardioscopy. A flexible and a rigid instrument 110° or 180° angled pericardioscopes (Storz®) were introduced. Videodocumentation (Sony Umatic) and photography (Ricoh) were performed. Representative images are demonstrated in Figure 2A and B.

**Epicardial Biopsies**

In all cases, an ACS 14 safety wire was introduced in the pericardial sac to allow rapid reintroduction of a pigtail cath-

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**Figure 1** Diagnostic and therapeutic approach to pericardial effusion.
eter in case of perforation by the biotome and intrapericardial hemorrhage. Up to eight epicardial biopsies were taken with resterilizable biotomes (Schikumed) after selection of the biopsy site by pericardioscopy and x-ray control in biplane positions (posterior anterior and 90° left anterior oblique view). After the biopsy site was inspected by pericardioscopy, the next biopsy site was selected and biopsy was carried out. After the entire procedure, the pigtail catheter was left in place until cytologic and/or histologic examination of the samples had been carried out and the treatment strategy was decided upon. Representative histology is demonstrated in Figure 3A and B.

**Pericardial Biopsies**

Up to five pericardial biopsies were taken with biotomes from Schikumed, using established techniques.17 In principle, a 7F biotome sheath was advanced to the pericardial edge over a teflon guidewire until it reached the silhouette of the heart in the posterior anterior view. The wire was exchanged with the biotome. The biotome was then advanced and the branches were opened immediately after it had passed through the sheath.

**Patient Classification According to Investigative Methods**

When lymphocytes were the predominant cell population in the pericardial fluid, the patient was allocated to the lymphocytic autoreactive pericarditis group. Autoactive predominantly antibody positive effusions had to be positive for antimitoemmal antibodies (AMLAs) of the IgG and IgA isotype and cellularity in the fluid had to be minimal. Bacterial or fungal pericarditis was excluded from this analysis, as were patients with a positive PCR, in situ hybridization, or positive viral culture for one of the following cardiotropic agents: entero-, adeno-, cytomegalo-, Ebstein Barr- influenza virus, *borreliosa Burgdorferi* or *chlamydia pneumoniae*. Bacterial pericarditis was diagnosed when the pericardial fluid contained bacteriae or acid fast bacilli either in the immediate smear analysis or in long-term culture.

**Results**

**Analysis of the Pericardial Fluid**

Of 136 patients treated over a period of 9 years, the patients and their laboratory parameters from the pericardial effusion...
were selected for this comparative analysis from a registry on pericardial effusion undergoing pericardiocentesis. According to the criteria outlined in the “Patient” and the “Method” sections, either an autoimmune or a neoplastic effusion had to be present and confirmed by epicardial biopsy or cytology.

Selection was also made on the ground of available follow-up data for 12 months for the patients with autoimmune and for at least 3 months for the patients with neoplastic effusion. All patients in the autoimmune group received intrapericardial treatment with triamcinolone (n = 14). In the group with neoplastic pericardial effusion, tumor etiology was bronchus carcinoma in seven patients (six oat cell carcinoma, one epithelioid cell carcinoma), breast cancer in three, adenocarcinoma of the colon or the esophagus in two, ovarian carcinoma, Hodgkin’s lymphoma, and non-Hodgkins lymphoma in one patient each. All 15 tumor patients received cis-platin intrapericardially.

Female patients prevailed in the autoimmune pericardial effusion group; their ages ranged between 17 and 71 years, with the majority between the ages of 17 and 60 years. In the neoplastic effusion group, the opposite was the case: male preponderance and an emphasis on patients beyond 50 years of age (Table I).

Serious effusion prevailed in autoimmune disease, whereas in neoplastic disease hemorrhagic fluid was more commonly found. Elevated LDH, when compared with serum values, was found more frequently in neoplastic disease, as was monocytosis. In contrast, lymphocytosis in the effusion prevailed in the autoimmune group of patients (Table I).

**Macroscopic Evaluation by Pericardioscopy**

The following criteria were evaluated regularly: presence of fibrin threads or a fibrin network at the epicardium, neovascularization or vascular injections, and protrusion. Figure 2A demonstrates a fibrin layer on the otherwise smooth epicardial surface and the small lesion due to epicardial biopsy. Figure 2B demonstrates tumor-related protrusion in a case of bronchus carcinoma. As outlined in Table II, fibrin threads, neovascularization, or increased vascular injections were similarly found in both groups of patients, although it appeared that the neovascularizations were more pronounced in neoplastic disease (graded data not shown). Protrusions were limited to neoplastic disorders and to tuberculous pericarditis (data not shown) and did not show in autoimmune pericarditis. When compared, autoimmune effusion protrusions were the only specific and sensitive parameter to distinguish one group from the other.

**Comparative Analysis of Cytology, Epicardial Biopsy and Pericardial Biopsy**

To assess the value of cytology and epicardial and pericardial biopsy in both disease groups, the number of positive patients for each method was evaluated (Table III). A trend could be demonstrated that in neoplastic disease epicardial biopsy was more sensitive than analysis of the pericardial fluid by cytology. A representative histologic example of bronchus carcinoma is given in Figure 3A. Both methods proved to be complementary for the final diagnosis. In autoimmune pericarditis, the inflammatory infiltrate (Fig. 3B) was detected regularly in the epicardial biopsy and lymphocytes, or mononuclear cells were found to a similar extent in the pericardial fluid. Pericardial biopsy did not prove truly informative except in one patient with neoplastic disease.

**Intrapercardial Administration of Triamcinolone: Acute Effect and Recurrence Rate**

When viral etiology and neoplastic cells were excluded from the pericardial fluid or epicardial biopsy and the diagnosis of autoimmune (lymphocytic) pericardial effusion was

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### Table I Differentiation between autoimmune and neoplastic pericardial effusion by laboratory parameters

<table>
<thead>
<tr>
<th>Parameters/effusion</th>
<th>Autoimmune</th>
<th>Neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n)</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Female (n)</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>17–71</td>
<td>47–78</td>
</tr>
<tr>
<td>Serous fluid (%)</td>
<td>86</td>
<td>7</td>
</tr>
<tr>
<td>Hemorrhagic (%)</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>Leukocytes &gt;8000/mm³ (%)</td>
<td>21</td>
<td>87</td>
</tr>
<tr>
<td>Lymphocytosis (&gt;3500/mm³ or more than 50% of WBC positive)</td>
<td>64</td>
<td>13</td>
</tr>
<tr>
<td>Monocytosis (&gt;50% pos)</td>
<td>7</td>
<td>87</td>
</tr>
<tr>
<td>LDH &gt;300 IU (&gt;50% pos)</td>
<td>14</td>
<td>87</td>
</tr>
<tr>
<td>Antimyolemmal antibodies in pericardial effusion (%)</td>
<td>86</td>
<td>47</td>
</tr>
<tr>
<td>Anti-tumor antibodies in pericardial effusion (%)</td>
<td>93</td>
<td>80</td>
</tr>
<tr>
<td>PCR against cardiotropic viruses (entero-, adeno-, CMV, EBV)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCR against borrelia Burgdorferi</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* p < 0.01 by chi-square analysis using Yates’ correction factor.

Abbreviations: pos = positive, WBC = white blood count, LDH = lactose dehydrogenase, CMV = cytomegalovirus, EBV = Epstein-Barr virus, PCR = polymerase chain reaction.

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### Table II Differentiation between autoimmune and neoplastic pericardial effusion by pericardioscopy

<table>
<thead>
<tr>
<th>Parameters/effusion</th>
<th>Autoimmune (n = 14)</th>
<th>Neoplastic (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrin threads (%)</td>
<td>86</td>
<td>100</td>
</tr>
<tr>
<td>Vascular injections in epicardium (%)</td>
<td>64</td>
<td>93</td>
</tr>
<tr>
<td>Protrusions (%)</td>
<td>7</td>
<td>88*</td>
</tr>
</tbody>
</table>

* p < 0.01 by chi-square analysis using Yates’ correction factor.
One female patient developed tachycardia and demonstrated elevated CK (up to 894 IU) and lactate dehydrogenase levels (up to 2346 IU). At 3-month follow-up, 7 of the 15 patients had died. Recurrence of tamponade or a larger pericardial effusion was reported in none of them. In one of the eight patients surviving the 3-month follow-up, recurrence of a substantial large pericardial effusion necessitated a second pericardiocentesis and intrapericardial cis-platin instillation. After 12 months, one patient with breast cancer and the two patients with Hodgkin’s and non-Hodgkin’s lymphoma had survived without need for another pericardiocentesis. Mortality and recurrences are outlined in Table IV. The 80% mortality in neoplastic pericardial effusion after 6 months clearly shows that the spreading of pericardial tumor is a late feature in terminal neoplastic disease.

### Discussion

It is obvious that assessment of the pericardial fluid allows a clear-cut differentiation between neoplastic and idiopathic pericardial effusion by cytology. The yield is improved by epicardial biopsy. Both methods, when applied alone or, even better, when carried out together can establish a firm diagnosis. This specific diagnosis permits specific intrapericardial anti-neoplastic treatment with cis-platin in high concentrations in the pericardial space. Although 80% of the patients did not survive 6 months due to tumor progression at other locations, the recurrence of a life-threatening effusion was very unlikely and thus found in one patient only. In contrast to systemic treatment, local application of cis-platin is well tolerated. Therefore, in neoplastic effusion, intrapericardial cis-platin application is the treatment of choice.

The entity defined here as autoimmune pericardial effusion probably comprises idiopathic pericardial effusion in many previous publications of other authors too numerous to be quoted here. This is primarily due to the fact that advanced molecular diagnostic methods such as PCR for microbial RNAs or DNAs, or immunohistochemistry for the characterization of epicardial infiltrate or for immunoglobulin binding in situ, or for anticardiac antibodies in the pericardial fluid have not been available. When viral persistence in the epicardium and the pericardial fluid is not detected, intrapericardial instillation of a crystalloid long-lasting triamcinolone suspension can be applied safely. From our experience, 1 g triamcinolone i.p. will exert its local effect for at least 4 to 6 weeks. It prevents

### Table III

**Differentiation between autoimmune and neoplastic pericardial effusion by cytology and epicardial and pericardial biopsy (% positive)**

<table>
<thead>
<tr>
<th>Parameters/effusion</th>
<th>Autoimmune (n = 14)</th>
<th>Neoplastic (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid: &gt; 3500 lymphocytes/mm³</td>
<td>64</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fluid: AMLAs positive for IgG, IgM, and IgA</td>
<td>86</td>
<td>27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytology diagnostic for inflammation or malignancy, respectively</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>Epicardial biopsy diagnostic for inflammation or malignancy</td>
<td>93</td>
<td>80</td>
</tr>
<tr>
<td>Pericardial biopsy diagnostic for inflammation or malignancy</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>PCR in fluid or biopsy positive for enteroviral RNA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCR in fluid or biopsy positive for CMV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCR in fluid or biopsy positive for EBV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCR in fluid or biopsy positive for <em>borrelia Burgdorferi</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.01 by chi-square analysis using Yates’ correction factor.

**Abbreviation:** RNA = ribonucleic acid. Other abbreviations as in Table I.

confirmed, 1 g triamcinolone in a crystalloid suspension was given intrapericardially for 24 h over the pigtail catheter which had been left in place after pericardiocentesis for the 24 h needed for this analysis.

Intrapericardial triamcinolone was tolerated well by all patients. Intermittent glucose intolerance with blood glucose levels up to 215 mg% was seen in three patients, in whom diabetes was not known previously. Assessment after 3 months gave a recurrence of the effusion in one patient and after 12 months in two patients (14%). One of the two patients had received colchicine 0.5 mg 3 times per day orally, the other patient was treated with a nonsteroidal antiphlogistic (ibuprofen) for 3 months. After 3 months there was recurrence in the one patient (7%) despite additional treatment with colchicine, and after 12 months their was recurrence after colchicine had been stopped previously in both patients. In one of these patients, a second pericardiocentesis and triaminoloneacetate administration intrapericardially had to be performed, and prolonged azathioprin and prednisone treatment was given orally thereafter. The patient with the second recurrence in the 12-month follow-up responded well to oral colchicine. Mortality and recurrences are outlined in Table IV.

### Intrapericardial Administration of Cis-Platin:

#### Acute Effect and Recurrence Rate

Of the 15 patients treated with 50 mg in 100 ml phosphate buffered saline intrapericardially, 14 tolerated the treatment well. One female patient developed tachycardia and demonstrated elevated CK (up to 894 IU) and lactate dehydrogenase levels (up to 2346 IU). At 3-month follow-up, 7 of the 15 patients had died. Recurrence of tamponade or a larger pericardial effusion was reported in none of them. In one of the eight patients surviving the 3-month follow-up, recurrence of a substantial large pericardial effusion necessitated a second pericardiocentesis and intrapericardial cis-platin instillation. After 12 months, one patient with breast cancer and the two patients with Hodgkin’s and non-Hodgkin’s lymphoma had survived without need for another pericardiocentesis. Mortality and recurrences are outlined in Table IV. The 80% mortality in neoplastic pericardial effusion after 6 months clearly shows that the spreading of pericardial tumor is a late feature in terminal neoplastic disease.

### Table IV

**Recurrence and mortality of autoimmune and neoplastic pericardial effusion after intrapericardial drug treatment**

<table>
<thead>
<tr>
<th>Parameters/pericardial effusion</th>
<th>Autoimmune (n = 14)</th>
<th>Neoplastic (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-month recurrence rate (%)</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>12-month recurrence rate (%)</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>3-month mortality rate (%)</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>6-month mortality rate (%)</td>
<td>0</td>
<td>80</td>
</tr>
</tbody>
</table>
Recurrence of symptoms and effusions very effectively but with serious side effects. Under these conditions, intrapericardial treatment will become the treatment of choice in inflammatory pericardial and perhaps myocardial disorders.

**Perspectives**

These two examples of local drug therapy are only prototypes of future treatment strategies for pericardial, myocardial, and even coronary disorders. This study could be carried out only when sufficient pericardial effusion was present to permit pericardial puncture without risking hemorrhage.

With new devices on the horizon, such as the PerDucer* (Comedicus), which will allow pericardial puncture without any effusion to be present due to the suction exerted on the pericardial layer, a completely new avenue in the local pericardial and myocardial drug treatment has been opened. Local anti-inflammatory treatment in myocarditis, intrapericardial antineoplastic treatment in small effusion, i.e. application of growth hormone in patients with heart failure and of cytokines and mediators of angiogenesis will become routine methods in the very near future. Local drug treatment has the unique advantage of little systemic side effects, thus improving the quality of the patients' lives and of a high concentration at the site of application, where it is really needed.

**Acknowledgment**

The authors acknowledge the kind support given by the departments of the following universities: The National Heart Hospital in London, Department of Cardiac Pathology (Prof. Dr. E. Olsen); the Department of Pathology (Direktor: Prof. Dr. Moll) and the Department of Hematology (Direktor: Prof. Dr. E. Olsen); the Department of Pathology (Direktor: Prof. Dr. Moll) and the Department of Hematology (Prof. Dr. Kochsiek K); the Department of Cardiac Pathology (Prof. Dr. E. Olsen); the Department of Pathology (Direktor: Prof. Dr. Moll) and the Department of Hematology (Direktor: Prof. Dr. E. Olsen); the Department of Pathology (Direktor: Prof. Dr. Moll) and the Department of Hematology (Prof. Dr. Kochsiek K) and report of the first 50 cases.

**References**

Efficient in Vivo Catheter-Based Pericardial Gene Transfer Mediated by Adenoviral Vectors

KEITH L. MARCH, M.D., PH.D.,* MICHAEL WOODY, M.S., KHAWAR MEHDI, M.D., DOUGLAS P. ZIPES, M.D., MARK BRANTLY, M.D.,† BRUCE C. TRAPNELL, M.D.‡

Krannert Institute of Cardiology, Indiana University School of Medicine; *R. L. Roudebush Veterans Administration Medical Center, Indianapolis, Indiana; †Pulmonary Branch, National Institutes of Health, Bethesda, Maryland; ‡Division of Pulmonary Biology, Children’s Hospital Medical Center, Cincinnati, Ohio, USA

Summary: Adenoviral vectors are promising agents for a number of in vivo gene therapy applications including diseases of the heart and coronary vessels. Efficient intravascular gene transfer to specific sites has been achieved in occluded vessels, but otherwise is hampered by the effect of blood flow on localized vector uptake in the vessel wall. An alternative delivery approach to coronary arteries is the expression of diffusible gene products into the pericardial space surrounding the heart and coronary arteries. However, in vivo pericardial access is comparatively difficult and has been limited to surgical approaches. We hypothesized that efficient adenovirus-mediated gene expression in pericardial lining mesothelium could be achieved by transmyocardial vector delivery to the pericardium. To evaluate this concept, a hollow, helical-tipped penetrating catheter was used to deliver vector-containing fluid directly into the intrapericardial space. The catheter was introduced percutaneously in anesthetized mongrel dogs, advanced into the right ventricle, and the tip passed through the apical right ventricular myocardium under direct radiographic visualization until the open end of the catheter tip resided in the intrapericardial space. Adenoviral vectors expressing either nuclear-localizing beta-galactosidase, cytoplasmic luciferase, or secreted human α1AT reporters (Av1nBg, Av1Lu, or Av1Aa, respectively) were instilled through the catheter into the intrapericardial space. Three days later the animals were sacrificed and reporter gene expression was evaluated in pericardium, epicardium, and multiple other tissues. In animals receiving Av1nBg, beta-galactosidase activity was evident in most of the pericardial lining endothelium, up to 100% in many areas. In animals receiving Av1Lu, luciferase reporter activity was abundant in pericardial tissues, but near-background levels were observed in other organs. In animals receiving Av1Aa, human α1AT was abundant (16–29 mg/ml) in pericardial fluid, but was undetectable in serum. All animals tolerated the procedure well with no electrocardiographic changes and no clinical sequelae. These observations demonstrate highly efficient adenovirus vector delivery and gene transfer and expression in the pericardium and support the feasibility of localized gene therapy via catheter-based pericardial approaches. We suggest that the pericardial sac may serve as a sustained-release protein delivery system for the generation of desired gene products or their metabolites for diffusion into the epicardial region.

Introduction

Heart disease is one of the most important clinical problems in humans and is a leading cause of death and morbidity. Adenovirus-mediated gene transfer to the myocardium and vasculature is a promising new treatment approach for some of these diseases in humans and offers a method to test specific hypotheses regarding the function of certain genes within cardiac and vascular tissues in animal models. Myocardial ischemia due to coronary insufficiency is a disease for which gene therapy may be useful, and one potential therapeutic approach could be to enhance collateral coronary circulation through induction of coronary angiogenesis. Recent studies have also suggested the feasibility of augmenting angiogenesis in models of peripheral ischemia by direct arterial gene transfer.1
Gene transfer to the vascular wall has been approached using a variety of vectors including retroviruses, DNA-liposomes, and adenovirus vectors, as well as by reimplantation of genetically modified vascular cells. Although adenovirus appears to be the most efficient of these vectors in vivo, high-frequency transduction has been accomplished in the vasculature only by isolation of a vascular segment for 20 to 45 min to allow for vector uptake into the cell of the vascular wall. Poloxamer 407 offers one possible strategy to reduce this requirement for a prolonged contact time by increasing the apparent adenovirus vector transduction rate by as much as 30 to 100 fold.

Another hurdle for localized therapeutic delivery to specific intravascular sites of disease is the difficulty in achieving high-efficiency local gene delivery without significant systemic delivery to many other sites. This difficulty is due to rapid blood flow and the finite time required for efficient vector uptake. Intravascular administration has been associated with extensive systemic gene transfer due to circulation of vector beyond the target tissue. Myocardial gene transfer has been performed using a variety of methods, including the delivery of adenovirus by arterial infusion or direct myocardial injection. Techniques employing direct intramyocardial injection limit systemic vector spread, but also restrict the area of cardiac transduction or grafting to only a few millimeters surrounding the injection site. Clinically significant levels of myocardial gene expression may require an approach that allows for more widespread transduction.

We hypothesized that an effective alternative delivery strategy would be catheter-based local gene transfer to pericardial mesothelium and high-level, localized expression of a potentially therapeutic, diffusible protein into the subepicardial interstitium and pericardial fluid compartment surrounding the heart and coronary arteries. To test this hypothesis, we employed a percutaneous approach using a hollow, helical-tipped catheter positioned transmurally across the right ventricular wall to deliver adenoviral vectors to the intrapericardial space. The results demonstrate virtually complete transduction of the parietal pericardium and, to a slightly lesser extent, the epicardium, with minimal to no systemic delivery. This strategy could have significant implications for gene therapy applications for cardiovascular disease.

Materials and Methods

Adenoviral Vectors

Replication-deficient recombinant E1-deleted adenovirus vectors AdInBg, Ad1Lu, Ad1Aa, encoding either a nuclear-targeted histochemical reporter (beta-galactosidase (β-gal)), a readily quantifiable cytoplasmic reporter (firefly luciferase), or a readily quantifiable secreted protein reporter (human α1-antitrypsin (α1AT)), respectively, were constructed previously and vector stocks were prepared, quantified, and stored as previously described.

Animals

Adult mongrel dogs (n = 11) were studied under protocols approved by the Institutional Animal Care and Use Committee, Indiana University School of Medicine, in accordance with the “Guide for the Care and Use of Laboratory Animals” [Department of Health and Human Services, publication No. (National Institutes of Health) 86-23, revised 1985]. Animals were maintained on a normal diet before and after vector delivery. Both surgical (n = 4) and percutaneous transeptal (n = 7) approaches were employed to evaluate pericardial gene transfer. Both procedures were performed under general anesthesia with thiopental-sodium (25 mg/kg). Following induction, animals were intubated and ventilated with oxygen containing 2% isoflurane for maintenance of anesthesia.

Percutaneous Delivery Approach

Percutaneous deliveries were performed using a hollow, helical-tipped catheter designed for controlled penetration into or through the myocardium during fluoroscopic visualization. Following placement of a 7 French sheath into the right jugular vein, a catheter was placed through the sheath and advanced under fluoroscopic guidance into the right ventricle to the cardiac apex, with the catheter tip directed inferiorly. An infusion of saline through the delivery lumen was maintained at 0.5–1 cc/min throughout this procedure in order to avoid clogging of the helical penetration tip with blood elements. Upon firm contact with the myocardium, the catheter tip was advanced through the myocardium using a gentle turning motion. After advancement over several mm, hand infusion of a 2:1 meglumine/normal saline mixture was initiated and contrast location was monitored fluoroscopically. Successful intrapericardial tip placement was identified by accumulation of contrast in the pericardium, at which point the catheter was fixed in position and flushed with 2 ml of saline prior to delivery of suspended vector. Vector suspension (1–5 × 10^10 plaque forming units) was then delivered in 8 ml of Dulbecco’s modified eagle’s medium (DMEM). Following delivery, final catheter position was confirmed by fluoroscopic visualization of a bolus of air instilled into the pericardial space, after which the catheter was removed.

Surgical Delivery Approach

As a control for vector delivery, adenovirus vector was also directly instilled into the pericardial sac using a surgical approach. Anesthetized animals were given 1.5 mg of pancuronium-bromide intravenously as a neuromuscular paralyzer. The chest was opened ventrolaterally in layers at the sixth intercostal space on the left side by electrocautery. The pericardial sac was grasped with an Allis clamp to dissect a small window through the pericardial fat pad. A circumferential suture was sewn into the pericardial sac, followed by insertion of a 24-gauge plastic catheter and tightening of the suture about the catheter. The adenovirus vector was diluted to 8 ml in DMEM (pH 7.4) containing 2% fetal bovine serum (FBS).
and was instilled through the catheter over several seconds. The suture was then tightened as the catheter was withdrawn. A chest tube was temporarily placed to expand the lung post-operatively and then withdrawn, and the wound in closed with sutures. A pleural suction trap filled with viricidal solution was used to prevent contamination. Animals were subsequently isolated and given free access to food and water.

**Analysis of Gene Expression**

Three days after vector delivery, animals were necropsied and gene transfer and expression in pericardial, epicardial, and other tissues were evaluated in animals (n = 7) receiving a mixture of Av1nBg and Av1Lu, using the histochemical chromogen substrate 5-bromo-4-chloro-3-indolyl-1-β-d-galactopyranoside (X-gal) as previously described. Following removal of the heart and sampling for luciferase assay (see below), the majority of the organ was rinsed in ice-cold phosphate-buffered saline (PBS) and fixed at 4°C for 4 h in a solution of PBS, 2% formaldehyde, and 0.02% glutaraldehyde. Tissues were then rinsed twice in PBS and stained for 4 h at 37°C in a buffered X-gal solution [3 mM K3Fe(CN)6, 3 mM K4Fe(CN)6, 2 mM MgCl2, and 1 mg/ml X-gal]. Successful gene transfer and expression of nuclear localizing beta-galactosidase was indicated by cells with blue nuclei.

Relative gene transfer to pericardium or potentially to other organs was evaluated by luminometry in dogs (n = 6) receiving Av1Lu using a quantitative cytoplasmic luciferase reporter. Briefly, samples of each tissue were removed and stored in ice-cold PBS prior to homogenization in lysis buffer (25 mM tris-phosphate pH 7.8, 2 mM dithiothreitol (DTT), 2 mM ethylenediaminetetraacetic acid (EDTA), 10% glycerol, 1% Triton X-100, 2 µg/ml aprotinin, 2 µg/ml leupeptin, 0.75 mM phenylmethylsulfonyl fluoride (PMSF), in PBS. Homogenates were centrifuged at 4,000 rpm for 10 min at 4°C, and the resulting supernatants centrifuged for an additional 10 min at 14,000 rpm and 4°C. Duplicate 20 µl samples of the cleared supernatant were evaluated for luciferase enzymatic activity with an Opticomp II luminometer (Gem Scientific, Hamden, Connecticut, USA). A 100 µl aliquot of assay reagent [20 mM Tricine, pH 7.8, 1.07 mM (MgCO3) 4Mg(OH)2•5H2O, 2.67 mM MgSO4, 0.1 mM EDTA, 33.3 mM dithiothreitol, 270 µM coenzyme A, 470 µM D-luciferin, and 530 mM ATP] was added to each 20 ml sample, and light production was measured over a 10-s period using the microprocessor-controlled photon counter. Background luminescence values were subtracted from sample values to arrive at relative light unit (RLU) values from each sample. Using the Bradford method, protein assays were performed on aliquots of the same tissue extracts to permit expression of these results as RLU normalized for protein concentration.

The secretion of adenovirus vector-mediated reporter protein into the pericardial space was evaluated in dogs (n = 3) following intrapericardial delivery of the Av1Aa vector. The total volume of intrapericardial fluid found at necropsy was approximately 2–3 ml. This pericardial fluid was evaluated for human α1-antitrypsin content using a standard radial immunodiffusion assay which has demonstrated lack of cross-reactivity with the canine protein.

**Results**

**Feasibility of Pericardial Gene Delivery**

Percutaneous delivery of the adenoviral vectors to the pericardium and subsequent gene transfer and expression in endothelial lining cells was well tolerated clinically in all animals over the time evaluated in this study. Because of the helical nature of the penetrating wound through the ventricular wall, overt bleeding into the pericardial sac did not occur upon removal of the catheter, as evidenced by evaluation of pericardial fluid at necropsy. Specifically, there were no hemodynamic changes or clinical sequela at any time, and all animals were well and without complications at necropsy 3 days after vector administration. Twelve-lead electrocardiograms (ECGs), obtained before vector instillation (baseline), immediately after instillation and at necropsy, revealed no changes in ECG rhythm or morphology or findings consistent with pericardial inflammation or myocardial damage (data not shown). Intrapericardial delivery via the percutaneous approach was demonstrated by fluorographic imaging of instilled contrast media (Fig. 1). As a control, adenovirus vectors were also administered to the pericardium via a surgical approach with a pericardiotomy. All animals tolerated the surgical procedure well clinically.

![Fig. 1 Sequential fluorographic images, obtained during a percutaneous delivery procedure, from the right anterior oblique projection. (A) Cardiac silhouette, with the helix catheter in place transmurally in the right ventricular wall. The instillation of contrast had just begun at the time of angiography; a thin layer of contrast is seen outlining the cardiac edge, confirming pericardial loculation. This image is represented as a line drawing in (B) for clarity. (C) The same projection after the infusion of approximately 15 cc of a mixture of radiographic contrast and vector suspension, with a line representation in (D).](image-url)
Gene Transfer to Pericardial Surface Mesothelium

Following intrapericardial adenovirus vector delivery, X-gal staining (n = 7) revealed widespread adenovirus-mediated gene transfer and expression over much of the visceral (epicardial) and parietal pericardium (Fig. 2). Low power en face examination of both surfaces showed diffuse blue staining indicating transgene expression in most of the surface [shown for parietal pericardium (Fig. 2A)]. Epicardial staining was typically most intense in the areas overlying the atria and auricular appendages. Microscopic inspection of parietal pericardium en face revealed nuclear histochemical staining in nearly 100% of cells in many areas, demonstrating efficient transduction of the mesothelial cell lining (Fig. 2B). Transgene expression was limited to the single cell layer immediately lining the pericardial space on the surface of the parietal pericardium (Fig. 2C) as well as the epicardium (Fig. 2D). These findings were not appreciably different between animals following percutaneous vector delivery and following surgical vector delivery, as also described previously.23

To estimate the ratio of infectious vector particles to target cells lining the pericardial space, for example, the multiplicity of infection (MOI), the pericardial and epicardial surface cell density was examined microscopically and the cell density was determined to be approximately $3 \times 4 \times 10^6$ cells per cm$^2$. Using a value of 132 cm$^2$ for the exposed intrapericardial surface area in dogs,24 the MOI was calculated to be about 20–100 pfu/cell assuming even spread of the vector in the pericardial sac.

Percutaneous Catheter-Mediated Adenovirus Delivery Is Localized to the Pericardium

To evaluate the relative amount of gene transfer locally into pericardial tissues versus systemically into noncardiac tissues following percutaneous vector delivery, Av1Lu was administered to dogs (n = 6). This particular vector was used because the luciferase enzyme reporter is confined to the cytoplasm of the gene-targeted cell and luciferase transgene expression is accurately and readily quantifiable in tissues by luminometry.25 Evaluation of cardiac tissues at several sites as well as lung, spleen, liver, and periaortic lymph nodes (n = 6) demonstrated that gene transfer was highly localized to the pericardium (Fig. 3). As expected from the X-gal staining results after the AvInBg vector administration, gene transfer and expression was highest in the parietal pericardium and epicardium, but markedly reduced by 500 to 1000-fold in endocardial and noncardiac tissues (Fig. 3). Thus, gene transfer after percutaneous, transventricular, helical-tipped catheter vector delivery is highly localized and targeted to pericardial mesothelial cells. Similar expression levels and localization were found 7 days following administration of AvInBg and Av1La (n = 1) with pericardial luciferase expression of 258,800 light U/mg protein.

![Fig. 2](image-url)

**Fig. 2** (A) Parietal pericardium stained with X-gal following in vivo exposure to the Av1nBg vector (16 $\times$ original magnification); punctate blue staining demonstrating the presence of transgene expression over the surface of the parietal pericardium. (B) Microscopic inspection of parietal pericardium en face (400 $\times$), demonstrating high-frequency transduction of the mesothelial cell lining. (C) Cross-sectional view of the parietal pericardium (250 $\times$); and (D) cross-sectional view of the epicardium following Av1nBg exposure, stained with X-gal as well as hematoxylin and eosin to demonstrate tissue morphology (250 $\times$).

![Fig. 3](image-url)

**Fig. 3** Cardiac and extracardiac distribution of luciferase expression. Tissue sampling was performed from numerous sites to permit evaluation of the amount of systemic vector distribution and concomitant gene expression found subsequent to the intrapericardial installation. Expression in various tissues is indicated. Luciferase activity given as relative light units/mg tissue protein (RLU/mg) normalized per 10$^7$ pfu administered. The asterisks indicate a mean luminometry of < 1000 RLU/mg. LV = left ventricular.
Adenovirus-Mediated Protein Expression into the Intrapericardial Compartment

To evaluate the efficiency of adenovirus-mediated delivery of a diffusible protein into the intrapericardial lining fluid, Av1Aa was administered to dogs (n = 3) via the percutaneous catheter route. This vector expresses human α1-antitrypsin, which is distinguishable from canine α1-antitrypsin by ELISA. An equal amount (10^9 pfu) of Av1Lu was coadministered with Av1Aa in each animal to permit parallel quantification of local and potential systemic gene transfer. Three days after administration, animals were evaluated for human α1-antitrypsin by ELISA in pericardial fluid and serum. At necropsy, approximately 2 to 3 mL of pericardial fluid was detected for each animal. Human α1-antitrypsin protein levels in pericardial fluid were 15, 19, and 26 µg/ml, respectively, in the three animals. However, no human α1-antitrypsin was detectable in the serum.

Discussion

Although gene therapy for cardiovascular disease is promising, especially with high-efficiency in vivo vectors such as adenovirus, localized delivery to the heart and vessel wall remains a challenge. Previous attempts to deliver genes to vessels or myocardium have generally been based on surgical isolation of a vessel or direct myocardial injection. While these approaches limit systemic spread, they are not yet entirely satisfactory for clinical application in humans. Direct instillation of adenoviral vectors into the intrapericardial space from either percutaneous catheter or surgical pericardiotomy approaches demonstrated very high efficiency gene transfer to the pericardial mesothelium. Gene transfer was localized to pericardial tissues with very little transduction of extracardiac tissues. This study thus demonstrates the feasibility of using a catheter-based approach to the pericardium as a route for adenovirus-mediated cardiac gene transfer, analogous to its use for targeted drug delivery.26–28 These results also demonstrate for the first time the possibility of pericardial gene transfer as an approach to sustained-release protein delivery, generating sufficiently high concentrations of desired gene products or their metabolites to result in diffusive transport into the epicardial region to an extent sufficient to produce therapeutic biologic effects.

Localized Gene Transfer to the Pericardium

The high transduction efficiency observed for the pericardial mesothelial cells is likely due to prolonged vector confinement within the pericardial space. Insofar as the vector particles are sufficiently large (100 nm in diameter) to render transpericardial diffusion minimal, the particles are restricted from systemic distribution and have no apparent route of elimination except that of uptake into the mesothelial lining cells. Other studies have evaluated a variety of enclosed cavities and potential spaces as targets for transduction, including the syn-ovial capsule,29 biliary system,30 intrapleural cavity,31 the intrathecal space,32 the intracranial cavity,33 and the pericardial space, using a surgical approach.34 Gene transfer to the peritoneal cavity has been explored as a method of localized gene transfer for systemic protein delivery.34 In this case, vector-derived human α1-antitrypsin was measurable in the systemic circulation at levels as high as 3.4 µg/ml.34 Systemic delivery in this case is presumably related to the relatively high surface area of the mesothelial lining of the peritoneum, approximately 2 m², consistent with the common clinical use of the peritoneal cavity for systemic solute or fluid transfer in the context of peritoneal dialysis. In contrast to these findings in the peritoneum, the current study shows high local expression of α1-antitrypsin in the absence of measurable circulating levels. This distinction likely reflects the significantly reduced pericardial surface area and possibly a difference in the intrinsic diffusive exchange properties of these two membranes.

Implications for Human Gene Therapy

The measured average value of 20 µg/ml in pericardial fluid found for α1-antitrypsin is higher than that found in several studies of systemic α1-antitrypsin gene transfer,3, 35 possibly as a consequence of the highly efficient transduction as well as localized secretion. This level is significantly greater than the levels required for many growth factors to exhibit physiologic effects. This suggests that the pericardial sac might potentially function as a sustained-release protein delivery system, generating sufficiently high concentrations of desired gene products or their metabolites to result in diffusive transport into the epicardial region to an extent sufficient to produce potentially therapeutic biologic effects. Such transport of macromolecules into the epicardial region has been measured and is consistent with observations of enhanced angiogenesis occurring in response to the epicardial placement of polymeric sustained release matrices containing basic fibroblast growth factor (bFGF).38 In addition, efficient diffusion through the visceral pericardium has been demonstrated and appears to be the primary mechanism by which large amounts of atrial natriuretic peptide are conveyed from subepicardial atrial cardiomyocytes into pericardial fluid;39 this fluid may in turn play a role as a physiologic reservoir for this and other endogenous compounds such as prostaglandins and peptide growth factors,39 much as is proposed for vector-encoded substances. Intrapericardial drug delivery has been described for several comparatively small compounds, including digoxin, lidocaine,40 amiodarone26 as well as other antiarrhythmic agents, and chemotherapeutic compounds.41 Although these studies have successfully demonstrated delivery, single-dose administration of most agents might not necessarily bring about a prolonged therapeutic effect due to the loss of agents from the pericardial compartment. In contrast, transfer of genetic material into cells lining the pericardium for subsequent protein expression provides one method for sustaining the effects resulting from a single instillation. A number of proteins may be suggested as candidates for such delivery, with potentially therapeutic approaches including the delivery of genes encod-
ing angiogenic proteins to enhance the natural process of collateral vessel formation in response to ischemia. The collateralizing effects of repetitive or sustained dosing protocols of vascular endothelial growth factor (VEGF) and bFGF proteins, as well as others, have been described in the context of multiple animal models of ischemia.42–45 The approach described here offers an alternative to delivery of these agents without the necessity for repetitive dosing or the implantation of sustained-release matrices.

Other potentially therapeutic candidates for pericardial expression include factors to promote vasodilation and smooth muscle quiescence, such as nitric oxide synthase (NOS) isoforms to enhance local NO production, or prostaglandin synthase to increase intrapericardial prostacyclin content. Locally enhanced expression of such genes might represent an antiangiogenic or antiinflammatory strategy of prolonged duration. In a similar fashion, it may be speculated that local synthesis of neuroactive peptides or other substances could act to modulate nerve conduction, thus affecting arrhythmogenesis or pain perception; and cardiotonic peptides have been described which might conceivably function to enhance myocardial contractility in a sustained fashion.

Finally, this study represents an initial description of a feasible and effective percutaneous approach for instillation of material into a normal pericardial space using a helical needle-tipped catheter. This method appears to be reasonably safe with no evidence of adverse sequelae seen over several days following the procedure. We suggest that percutaneous transluminal intrapericardial delivery using a range of devices may permit the minimally invasive instillation of nongenetic as well as genetic therapeutic agents with relative ease and safety for a variety of potential indications.

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References


Initial Clinical Experience with PerDUCER® Device: Promising New Tool in the Diagnosis and Treatment of Pericardial Disease

PETAR M. SEFEROVIC, M.D., PH.D., FACC, FESC, ARSEN D. RISTIC, M.D., M.SC., RUŽICA MAKSIMOVIC, M.D., M.SC., PREDRAG PETROVIC, M.D., PH.D., MIODRAGO OSTOJIC, M.D., PH.D., FACC, FESC, SLAVKO SIMEUNOVIC, M.D., PH.D., FACC, DANIELA ZAMAKLAR, M.D., DEJAN SIMEUNOVIC, M.D., *DAVID H. SPODICK, M.D., D.SC., FCCP, FACC

Institute for Cardiovascular Diseases of the University Medical Center of Serbia, Belgrade, Yugoslavia; *Cardiology Division, St. Vincent Hospital and the Department of Cardiology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

Summary

Background: The idea to enter the normal pericardial sac safely was unrealistic until recently. The development of a novel instrument (PerDUCER® pericardial access device) for percutaneous access to the pericardium could potentially have a significant impact, not only on patients with pericardial diseases but even more, or primarily, on diagnosis and treatment of myocardial and coronary disease and arrhythmias.

Hypothesis: The overall objective of the present study was to evaluate the feasibility and safety of the percutaneous pericardial access with PerDUCER in patients with pericardial disease, and to analyze our initial experience with this new technique, with particular emphasis on sequential procedural steps.

Methods: The device was studied in five patients with pericardial disease (two men, mean age 50.4 years, range 30–68, four with normal body mass index). The procedure consists of two distinct techniques: (1) access to the mediastinal space, and (2) pericardial capture, puncture, and insertion of the guidewire. Access to the mediastinal space includes the introduction of a blunt cannula, a 0.038 guidewire, a dilator-introducer sheath set, and insertion of the PerDUCER device. Key points of the PerDUCER procedure are as follows: introduction of the blunt cannula without resistance, placement of the dilator-introducer sheath at the upper third of the heart, systolic movements of the PerDUCER device, successful vacuum and capture of pericardium, puncture and introduction of the intrapericardial guidewire.

Results: Access to the mediastinal space was accomplished in four of five patients, as were pericardial capture and probably puncture. However, despite numerous successful captures and probably punctures of pericardium, we were not able to confirm introduction of the intrapericardial guidewire into the pericardial cavity in any of our patients (0/5). The procedure was very well tolerated in all patients (5/5). No major complications developed during the procedure, bearing in mind that the intrapericardial placement of the guidewire was not achieved. Minor complications included pain at the dilator-introducer sheath entry site (5/5) and mild transient fever (2/5).

Conclusions: According to the present experience, we believe that, with minor modifications, the PerDUCER device could be successfully implemented for pericardial entry in patients with pericardial disease. Further studies are needed to evaluate the feasibility and safety of this new instrument in patients with a normal pericardium. This could open a most exciting spectrum of possible implementations of the device in the future.

Key words: pericardium, pericardiocentesis, pericardial effusion, new devices

Introduction

Since a Spanish physician, Romero, first performed successful closed puncture of the pericardium in 1803, various approaches to the pericardium have been attempted.1 Pericardiocentesis became a routine procedure worldwide because of its excellent clinical efficacy and prompt lifesaving effect. Although reported by numerous investigators as convenient, low cost, and less troublesome than surgery, it has been associated with a significant risk of complications, higher than during cardiac catheterization and a number of invasive procedures.2–4 The probability of both success and complications of pericardiocentesis are mainly related to the volume, location, and loculation of the pericardial effusion. The procedure is most likely to be successful when performed in patients with an echocardiographically free space of 10 mm or more, and with anterior effusions.5
Until recently, the idea of safely entering the normal pericardial sac or pericardium with minimal effusion was only a dream. The potential diagnostic and therapeutic implications of such an approach have fascinated cardiologists for more than a century. A new concept in approaching pericardial space has been developed, and a novel instrument (PerDUCER® Pericardial Access Device, Comedicus Inc., Columbia Heights, Minnesota) for percutaneous access to the pericardium was introduced. After meticulous experimental evaluation of this device in the animal setting, it became clear that this technique could potentially have a tremendous impact not only on patients with pericardial diseases, but even more, or primarily, for diagnosis and treatment of myocardial and coronary disease and arrhythmias. If this technology meets expectations, the pericardial space may be employed to apply therapeutic agents directly on the epicardial surfaces of the heart and coronary arteries. Such implementation could provide more effective and extended drug action, no agent loss into the circulation, and few side effects.

Before routine use of this device in humans, various technical aspects of its practical application need to be clarified. Therefore, the overall objective of the present study was to evaluate the feasibility and safety of percutaneous pericardial access with the PerDUCER device in patients with pericardial disease and to analyze our initial experience with this new technique, with particular emphasis on sequential procedural steps.

### Methods

#### Patients

The baseline characteristics of the patients in whom the PerDUCER device was employed are demonstrated in Table I. In June and July 1998, five patients (two men, mean age 50.4 years, range 30–68), four with normal body mass index, underwent the pericardial access procedure using the PerDUCER device. The mean duration of pericardial disease was 55 days (range 14–90 days). Two patients had idiopathic pericardial effusion, in another two pericardial effusion was due to an acute viral illness, and one patient suffered from neoplastic pericardial disease. Pericardial effusion assessed by echocardiography anterior to the right ventricle (RV) ranged from 0.8 to 2.2 cm (mean 1.6 cm). Four patients had no previous pericardial procedures, while the patient with a neoplastic effusion had emergency pericardiocentesis necessitated by cardiac tamponade (600 ml of hemorrhagic effusion evacuated, 32 days before the PerDUCER procedure). Subsequently, pericardial effusion recurred in this patient (range 0.6–0.8 cm). Therefore, at the time of the PerDUCER procedure, all five patients had moderate to large pericardial effusions, without signs of cardiac tamponade. In two patients pericardial thickness was measured by both transesophageal echocardiography (TEE) and magnetic resonance imaging (MRI). The values obtained were comparable for TEE and MRI and were 2–3 mm and 1.7–2 mm, respectively. Magnetic resonance imaging measurement of the pericardium thickness in patient No. 5 is shown in Figure 1.

### Table I

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<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>BMI (kg/m²)</th>
<th>Duration of pericardial disease (days)</th>
<th>Etiology</th>
<th>Pericardial effusion in front of RV by echo (cm)</th>
<th>Pericardial thickness by TEE (mm)</th>
<th>Pericardial thickness by MRI (mm)</th>
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**Abbreviations:** F = female, M = male, BMI = body mass index, RV = right ventricle, TEE = transesophageal echocardiography, MRI = magnetic resonance imaging.

Fig. 1 Magnetic resonance imaging measurement of the pericardial thickness in Patient No. 5 revealing 2 mm in front of the right ventricle (black arrow).
The PerDUCER Device: Features Related to Clinical Application

The device is essentially a sheathed needle, 35.6 cm (14") long, constructed of a 21 gauge stainless steel introducer needle located inside a stainless steel sheath. The proximal end of the PerDUCER device consists of a thermoplastic handle capable of counterclockwise rotation, which controls radial and axial movement of the introducer needle. Furthermore, the handle has both markings for rotation of the introducer needle and an integrated female luer vacuum side port. At the distal tip of the instrument there is a viewing tube with a hemispherically shaped side-hole cavity, where the pericardium is captured by vacuum and tangentially punctured by the introducer needle.

The PerDUCER Device: A Comprehensive Overview of Feasibility

All procedures were performed in the cardiac catheterization laboratory. Patients were extensively informed about the potential benefits and risks of the procedure and were requested to sign a written consent form.

Before approaching the pericardium, right heart catheterization and RV angiography were performed to reveal the RV contour, especially its inferior border. Such an approach, in most cases of standard pericardiocentesis, prevents the puncture or damage of the RV. The pigtail catheter remains in the RV for occasional manual dye injections, which improves orientation during the procedure. Therefore, such an approach was also followed before the PerDUCER procedures.

A pericardial access procedure with the PerDUCER device consists of two distinct techniques: (1) access to the mediastinal space, and (2) pericardial capture, puncture, and insertion of the guidewire.

Access to the Mediastinal Space

After administration of local anesthesia (20 ml 2% lidocaine), a small stab wound incision was made, just below the xiphoid process in the median line. The blunt cannula was introduced substernally until the mediastinal space was entered. At this point, it was important to perform RV manual injections from the previously placed pigtail catheter to improve the orientation and avoid possible complications. The use of the left lateral angiographic view was mandatory for optimal visualization of the cannula advancement and relations to the RV. Usually, entry into the mediastinal space was with minimal resistance, and therefore, if higher resistance is felt, caution should be exerted. The direction of the puncture should be as parallel as possible with the sternum. After placement of the cannula in the mediastinal space, an 0.038 guidewire was subsequently advanced and could be seen to move freely in the mediastinal space.

Instead of inserting and positioning the dilator-introducer sheath over the guidewire in one stage as recommended, we felt that it is more appropriate to perform gradual, subsequent dilation of the tunnel created with 8, 10, 12, 14, 16, and 19F dilators. Moreover, placement of large dilators could be painful in some patients, and therefore 2 ml thalamonal intravenously (IV) (fentanyl 0.05 mg + droperidol 2.5 mg/ml) was applied. Step-by-step dilation prevented difficulties during introducer sheath placement, particularly in obese patients, as we encountered in two cases (2/5). Figure 2 demonstrates the proper position of the 19F introducer in the mediastinal space, with guidewire inserted. Nonetheless, the current introducer sheath probably needs to be stiffer in order to provide more pushing force and to avoid kinking or collapse. Furthermore, sheath radio-opacity needs to be improved to facilitate its positioning. When the tip of the dilator-introducer sheath was placed at the upper third of the heart (left lateral angiographic view), both the dilator and the guidewire could be withdrawn. At this point, we found it very useful to inject 50% diluted angiographic contrast through the sheath to visualize its position in the mediastinal space and relation to the heart, as shown in Figure 3. This maneuver proved useful in three (3/5) of our patients, allowing the operator to achieve the desired sheath position and eventually to optimize the pericardial access site.

After loading of the intrapericardial guidewire and connection with the vacuum syringe, the PerDUCER device was inserted into the introducer sheath and advanced under fluoroscopic control until contact with the pericardium. Rarely, advancing the device can be difficult due to bending, kinking, or collapse of the sheath, a phenomenon we experienced in one case (1/5). Placement of the distal tip of the PerDUCER device over the correct pericardial access site is perhaps one of the key points of the procedure.

Pericardial Capture, Puncture, and Guidewire Insertion

Proper contact of PerDUCER device and pericardium was confirmed by its movements simultaneously with heart beats. This was an important sign of successful progress of the pro-

![Fig. 2 Proper position of the 19F dilator (black arrow) in mediastinal space, with guidewire inserted. Pigtail catheter in right ventricle in place. Left lateral angiographic view.](image-url)
procedure and could be seen clearly from outside the chest. We observed this phenomenon in four of our patients (4/5). In achieving the optimal PerDUCER device position, it was essential to place the flattened surface of the viewing tube against the target pericardial site. Figure 4 reveals a correct device placement and its position in relation to the heart. After close contact with the pericardium was secured, a vacuum using the 20 ml syringe was drawn to capture a pericardial “bubble” into the hemispheric cavity of the viewing tube. A successful vacuum could be recognized as sustained tension on the syringe plunger, which we encountered in numerous instances in four of our patients (4/5).

Advancing the handle of the device moves the needle tangentially and punctures the pericardial “bubble” without risking of any damage. The puncture position of the needle can be seen easily on fluoroscopy, as depicted in Figure 5. After advancing the puncturing needle and the guidewire, the vacuum on the syringe is diminished or lost. The intrapericardial guidewire should then be inserted through the needle and placed into the pericardial space. The intrapericardial position of the guidewire is not easy to recognize, and therefore it should be verified in several angiographic views. When the guidewire is in the pericardium, the needle should be retracted and the PerDUCER device withdrawn. The intrapericardial guidewire can then be used to introduce other diagnostic or therapeutic devices.

All patients received a prophylactic antibiotic regimen (gentamycin 80 mg IV b.i.d., ampicillin 500 mg orally q.i.d. for 3 days) starting on the day of the intervention. Most patients could be discharged a day or two after the procedure.

Results

The most important procedural data are presented in Table II. Access to the mediastinal space was accomplished in four of five patients, as were pericardial capture and probably puncture. However, despite numerous successful captures and probable punctures of the pericardium, we were not able to confirm introduction of the intrapericardial guidewire into the pericardial cavity in any of our patients (0/5). Figure 5 illustrates a failed attempt of intrapericardial guidewire placement and its position in the mediastinal space.

The pericardial access procedure with the PerDUCER device was very well tolerated in all patients (5/5). No major complications developed during the procedure, keeping in mind that intrapericardial placement of the guidewire was not achieved. Minor complications included pain at the dilator-introducer sheath entry site (5/5) and mild transient fever (2/5). Pain was successfully treated with the above-mentioned IV analgesics.

Since two of five patients with a large pericardial effusion needed pericardiocentesis for diagnostic reasons, standard...
pericardial puncture was performed following the PerDUCER device study. The amounts of pericardial fluid obtained was 1200 and 700 ml.

Discussion

Cumulative experience with the PerDUCER device comprises animal experiments, studies on human cadavers, and clinical experience in patients undergoing cardiac surgery. Animal experiments were conducted mostly on pigs and were focused on procedural technique, hemodynamic considerations, the extent of myocardial and pericardial trauma, and therapeutic applications of the method.6 Studies on cadavers highlighted the technique performance within human anatomy and the potential technical drawbacks of the device.6 These studies are considered highly informative and successful, supporting the plans for clinical trials. Open-chest experience with the PerDUCER device has been conducted in eight patients, in whom the pericardium was captured, punctured, and the guidewire successfully inserted into pericardium without complications.10

One of the key points of the procedural success in interventional cardiology is a proper indication for the particular method. The PerDUCER device is designed for puncture of both diseased and healthy pericardium. In patients with pericardial effusions, the device may replace standard pericardiocentesis as a more efficient and safer method. However, as already pointed out, the first experimental and open chest studies were designed and performed in subjects with a normal pericardium, highlighting new, more attractive diagnostic and therapeutic indications, unknown before in human medicine.

In contrast to previous experience, in the present study the PerDUCER device was used in patients with pericardial effusion, in a closed chest setting, with local anesthesia, and in the cardiac catheterization laboratory. Therefore, the conditions of our study corresponded to circumstances in which the device should be applied most frequently or exclusively in the future. In analyzing every sequential procedural step, some of the key points can be identified, as shown in Table III.

The use of RV angiography at this very early stage of application of the instrument was very instrumental in avoiding complications. Because of the simple design of the PerDUCER device, it is obvious that for the trained interventional cardiologist the learning curve will be rather short, and for other cardiologists very acceptable.

Despite several advantages of the instrument, introduction and placement of the intrapericardial guidewire was not accomplished. The potential reasons for failure can be classified into three groups: insufficient number of patients, erroneous patient selection, and the need for minor technical improvements of the device.

No definite conclusions about a new technology may be based on experience with five patients. Furthermore, all patients in this study had pericarditis of significant duration (mean 55 days) and associated with effusion (mean 1.58 cm). Taking into account these factors, one can hypothesize that in this patient cohort the thickness and/or elastic properties of the pericardium were increased abnormally, with augmentation of its fibrotic component. To elucidate this issue, we measured the pericardial thickness in two patients by both TEE and MRI and obtained values that were within the normal range.11 Nonetheless, significant effusion can produce tension on the pericardium, which is a clear impeding factor for capture and puncture. The same is true for pericardial fat that can easily obstruct the hemispheric side-hole cavity of the viewing tube and prevent capture. In addition, under the conditions of a thick and stiff pericardium, the side-hole cavity is inadequately small in both size and depth to enable capture. For the same reason, the vacuum intensity may not be sufficient to provide capture, and other means for generating a stronger vacuum, including electrical suction, may be needed.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>PerDUCER procedure: Procedural data</th>
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<tbody>
<tr>
<td>Patient No.</td>
<td>Access to the mediastinal space</td>
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<tr>
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</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>4</td>
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<td>Yes</td>
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</table>

<table>
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<tr>
<th>TABLE III</th>
<th>Key points of the PerDUCER procedure</th>
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<tr>
<td></td>
<td>Introduction of the blunt cannula without resistance</td>
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<tr>
<td></td>
<td>Placement of the dilator-introducer sheath at the upper third of the heart</td>
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<td></td>
<td>Systolic movements of PerDUCER</td>
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<td></td>
<td>Successful vacuum and capture of pericardium</td>
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<tr>
<td></td>
<td>Puncture and introduction of intrapericardial guidewire</td>
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</table>
Conclusions

A new percutaneous pericardial access device, PerDUCER, was studied in five patients with pericardial disease. The procedure consists of two distinct techniques: (1) access to the mediastinal space, and (2) pericardial capture, puncture, and insertion of the guidewire. The access to the mediastinal space (the introduction of a blunt cannula, a 0.038 guidewire, a dilator-introducer sheath set, and the PerDUCER device) was successfully achieved in four patients. Pericardial capture and probable puncture were also accomplished in numerous instances in four patients, but despite this we were not able to confirm introduction of the guidewire into pericardial cavity in any of our patients. The procedure with PerDUCER device was very well tolerated in all patients (5/5). No major complications developed during the procedure, while minor complications included pain at the dilator-introducer sheath entry site (5/5) and transient fever. It is our impression that this procedure is as well tolerated as cardiac catheterization.

According to the present experience, we believe that with minor modifications the PerDUCER device could be successfully implemented for pericardiocentesis in patients with pericardial disease. Further studies are needed to evaluate the feasibility and safety of this new instrument in patients with a normal pericardium. This could open a most exciting spectrum of implementations for intrapericardial diagnostics and therapy in the future.

References

Minimally Invasive Access of the Normal Pericardium: Initial Clinical Experience with a Novel Device

MICHAEL P. MACRIS, M.D. AND STEPHEN R. IGO*

Division of Cardiovascular Surgery, Spring Branch Medical Center, Houston, and *CorMedics Corporation, Clear Lake Shores, Texas, USA

Summary: The pericardial space is being investigated as a reservoir for local drug delivery to the heart and coronary arteries. Intrapericardial drug delivery is currently limited because the pericardial space is normally small and difficult to access by standard pericardiocentesis without invasive surgery or risk of cardiac injury. Clinical trials are being conducted to evaluate a novel, minimally invasive, pericardial access device (PerDUCER®, Comedicus Inc., Columbia Heights, Minn.). As of October 26, 1998, 12 clinical trials have been completed on patients undergoing cardiac surgical procedures. In all patients, a stab incision was made 1” subxiphoid and a 17G angled cannula, with preloaded guidewire, was advanced into the mediastinal space. After cannula removal, a 19F sheath/dilator was inserted over the wire. In eight patients, a median sternotomy was performed and the position of the sheath over the anterior pericardium (PC) was visually verified. Four patients underwent a closed-chest, fluoroscopy-assisted procedure. In all patients, the PerDUCER was inserted into the chest, via the sheath, and positioned over the PC. The PC was captured by suction and a bleb was formed within a side-hole on the PerDUCER tip. A sheathed needle was advanced, puncturing the isolated bleb of PC. A guidewire was advanced through the needle into the pericardial space and the PerDUCER was removed. Guidewire insertion was successful in 10 patients (7 on first attempt, 3 on second) without adverse hemodynamic effects or arrhythmia. Other than the guidewire insertion site, there was no evidence of injury to the PC or the heart. These initial clinical trials suggest that the PerDUCER may provide safe, rapid and effective percutaneous insertion of a guidewire into the normal pericardial space.

Key words: intrapericardial access, pericardiocentesis device, minimally invasive

Introduction

The pericardial space has been proposed as a new route for local drug administration and device insertion to the heart and coronary arteries. Angiogenesis,1, 2 antithrombotic,3 antiarrhythmic,4, 5 cardioprotective,6 and percutaneous transluminal coronary angioplasty restenosis7 therapies are being investigated. Intrapericardial drug delivery has not been utilized for heart-specific treatments where the pericardium is normal because the pericardial space is small and very difficult to access by standard pericardiocentesis techniques without invasive surgery or risk of cardiac injury. This report describes the initial U.S. clinical trials of a novel, minimally invasive pericardial access device (PerDUCER), designed specifically to provide safe and effective access to the normal pericardium.

Materials and Methods

The PerDUCER (Comedicus Incorporated, Columbia Heights, Minn.) is a percutaneous, sheathed-needle, pericardial access device that is designed to avoid injury of the heart during pericardial catheterization. The device consists of a 21-gauge needle housed inside a 12 French stainless steel sheath tube 20 cm in length. One end of the sheath tube is bonded to a plastic handle with a side-arm port that is connected to a suction syringe. The proximal end of the needle passes through a seal in the handle and is attached to a rotating assembly, on the outside of the handle, which controls the rotational and axial movement of the needle. A Tuohy Borst adapter is bonded to the rotating assembly and connects to the needle. A 0.018” J-tipped guidewire is preloaded into the needle and sealed by the Tuohy adapter. The distal end of the sheath tube is bonded to a
plastic tube with a tapered end and a half-moon shape (18.5 French maximum OD). The flat portion of the tube, intended to be placed on the pericardial surface, has a hemispherical-shaped, 4.8 mm OD side-hole which communicates with the suction lumen and also contains the travel of the needle point. Inside this isolation well is where pericardial capture and needle puncture are accomplished. The PerDUCER has “P” and “I” markings located on the rotating assembly to denote proper needle position for pericardial “Puncture” and guidewire “Insertion,” respectively.

Clinical trials of the PerDUCER pericardial access device have been conducted on 12 patients undergoing cardiac surgery under a Non Significant Risk Protocol from the IRB (Phase I Safety Study, open-chest, and Phase II Efficacy Study, closed-chest). The clinical study protocols were approved by the Institutional Review Board of Spring Branch Medical Center, Houston, Texas. Informed consent was obtained from all patients prior to surgery. Inclusion criteria included patients with no history of prior cardiac or thoracic surgery (sternotomy or thoracotomy); no active cardiac ischemia or arrhythmia; no myocardial infarction within 72 h of study; and stable cardiac, neurologic, pulmonary, coagulation, and renal function.

PerDUCER insertion was performed following induction of anesthesia with continuous hemodynamic and electrocardiogram (ECG) monitoring. In all patients, a small stab incision was made approximately 1” below the xiphoid process. A 17-gauge curved blunt cannula, preloaded with a 0.038” J-tipped guidewire, was inserted subcutaneously and carefully advanced, with the cannula curve directed toward the posterior surface of the sternum, through the diaphragm, and into the anterior mediastinal space. The guidewire was advanced through the cannula and observed to move freely in the mediastinal space. The cannula was removed and a 19 French sheath/dilator was inserted over the mediastinal guidewire. The dilator was unlocked from the outer sheath and withdrawn along with the guidewire, leaving the introducer sheath located in the anterior mediastinal space.

In eight patients a median sternotomy was performed, with minimal retraction, and the position of the sheath over the anterior pericardial surface was visually verified (Phase I studies). Four patients underwent closed-chest, fluoroscopy-assisted PerDUCER placement (Phase II studies). In all patients, the PerDUCER was inserted into the chest, through the sheath, and oriented with its tip located in the mediastinal midline approximately at nipple level. The side-hole on the flat plane of the device tip was placed against the pericardial target site. Using gentle downward force, the PerDUCER was moved in a back-and-forth motion in order to dissect a plane under the pericardial fat pad with the tapered end of the device.

The PerDUCER was first set in the “Puncture” position (needle retracted, needle bevel up) by aligning the “P” on the rotating assembly with the pin on the handle (Fig. 1A). Pericardial capture was accomplished by pulling back on the plunger of the suction syringe, connected to the PerDUCER handle, until resistance to further withdrawal was obtained. As suction was maintained, a pericardial bleb was formed within the side-hole well, thus isolating the pericardium away from the epicardial surface of the heart. Pericardial puncture was accomplished by briskly pushing the rotating assembly forward and advancing the needle (Fig. 1B). The rotating assembly was rotated 180° fully counter-clockwise to position the needle bevel opening (pointing downward toward the heart) for insertion of the intrapericardial guidewire (Fig. 2A). Suction was no longer required with the syringe because the pericardium is trapped in the side-hole bleb chamber by the full excursion of the needle (Fig. 2A). The Tuohy adapter was loosened by turning counter-clockwise and the guidewire was advanced 15 to 20 cm into the pericardial space (Fig. 2B). Proper placement of the guidewire was verified by observation of a characteristic loop defined by the margins of the pericardial sac. The rotating assembly was pulled back, aligning the “I” marking with the pin on the handle, thereby retracting the needle within the sheath tube (Fig. 2B). The PerDUCER was slowly withdrawn through the introducer sheath, leaving the intrapericardial guidewire in place. Following PerDUCER removal the chest was opened. The location of the intrapericardial guidewire was verified and photographed, and a small section of pericardium, at the guidewire insertion site, was excised for histologic examination.

Fig. 1 Illustration of the PerDUCER pericardial access device showing its handle, suction syringe, and cross section (close-up) of tip, pericardium, and myocardium during suction capture of the pericardium (A) and pericardial puncture (B).
Results

As of October 26, 1998, 12 clinical trials have been completed on patients undergoing cardiac surgical procedures at Spring Branch Medical Center, Houston, Texas. The patients (8 men, 4 women) ranged in age from 49 to 80 years (mean 66), and had an average height of 172 cm and weight of 90 kg. Guidewire insertion was successful in 10 patients (7 on the first attempt and 3 on the second attempt) without adverse hemodynamic effects (stable heart rate, aortic pressure, pulmonary artery pressure) or arrhythmia. The two technically unsuccessful procedures were both closed-chest cases. For the first, the guidewire was believed to be properly positioned intrapericardially, as determined fluoroscopically, but the guidewire was dislodged during the performance of the sternotomy. For the second, the guidewire was extrapericardial and located in the pericardial fat pad. The total procedure time from subxiphoid cannulation to intrapericardial guidewire insertion, via the PerDUCER, was < 15 min. Other than the guidewire insertion site, there was no evidence of injury to the pericardium or heart.

Discussion

Knowledge of the pericardium dates back to the time of Galen (129–200 AD), the Greek physician and anatomist who gave the pericardium its name. The pericardial sac surrounds the heart like a glove enfolds a hand and the pericardial space is naturally fluid-filled. The normal pericardium functions to prevent dilatation of the chambers of the heart, lubricates the surfaces of the heart, and maintains the heart in a fixed geometric position. It also provides a barrier to the spread of infection from adjacent structures in the chest and prevents the adhesion of surrounding tissues to the heart. The normal pericardial space is small in volume and the fluid film within it is too thin to separate the heart functionally from the pericardium. When fluid is injected into the normal pericardial space, it accumulates in the atrioventricular and interventricular grooves, but not over the ventricular surfaces.

Pericardiocentesis, or puncture of the pericardium, is indicated for (1) withdrawal of pericardial fluid for the treatment of cardiac tamponade, (2) diagnosis of pericardial disease(s) by study of the pericardial fluid, and (3) infusion of therapeutic agents for the treatment of malignant effusion or tumors. Clinically, drugs that have been injected into the pericardial space include antibiotic (sclerosing) agents, antineoplastic drugs, radioactive compounds, and fibrinolytic agents. The pericardiocentesis procedure is conducted by experienced personnel in the cardiac catheterization laboratory with equipment for fluoroscopy and ECG monitoring. Electrocardiographic monitoring of the procedure using the pericardiocentesis needle as an electrode is commonly employed. Use of an echocardiographic transducer has also been utilized to guide the pericardiocentesis needle. Complications associated with conventional needle pericardiocentesis include laceration of a coronary artery or the right ventricle, perforation of the right atrium or ventricle, puncture of the stomach or colon, pneumothorax, arrhythmia, tamponade, hypotension, ventricular fibrillation, and death. The complication rates for conventional needle pericardiocentesis are increased in situations where the pericardial space and fluid effusion volume are small.

Intrapericardial drug delivery has, as yet, not been clinically utilized for heart-specific treatments where pericardial pathology is normal, because the pericardial space is small and very difficult to access without invasive surgery or risk of cardiac injury by standard needle pericardiocentesis techniques. Transvenous methods of pericardial catheterization have been investigated involving puncture of the right atrium with a needle-catheter assembly and puncture of the right ventricle with a helix-needle catheter. The major limitation of these methods, in contrast to the PerDUCER, is that the right atrial or ventricular wall is penetrated, which could lead to bleeding into the pericardial space. In addition,
these methods involve a bolus injection of drugs, rather than long-term delivery via an indwelling catheter.

Conclusion

The results from these initial clinical trials suggest that the PerDUCER may provide safe and effective percutaneous guidewire insertion into the normal pericardial space.

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Establishment of a Clinically Correlated Human Pericardial Fluid Bank:
Evaluation of Intrapericardial Diagnostic Potential

TONYA J. DICKSON, M.S.,* VIVEK GURUDUTT,† A. Q. NGUYEN, M.S., * K. KUMFER,*, W. MAXTED, *, JOHN BROWN, M.D., †
YOUSUF MAHOMED, M.D., † THOMAS SHARP, M.D., † THOMAS X. AUFIERO, M.D., † NAOMI FINEBERG, PH.D.* † KEITH L. MARCH, M.D., PH.D* ‡

*Krannert Institute of Cardiology, †Indiana University School of Medicine, and ‡R.L. Roudebush VA Medical Center, Indianapolis, Indiana, USA

Summary: The development of a clinically correlated human pericardial fluid bank and database is described. A unique feature of this registry is the availability of a large number of pericardial fluid samples for testing with respect to multiple factors and for correlation with angiographic findings and clinical syndromes expressed by the patients. The collection of data at the present time comprises frozen pericardial fluid samples obtained from patients who have undergone cardiac surgery; and historical, clinical, and laboratory data obtained from the patient records. Nearly 400 samples have been stored and analyzed thus far, with sample entry continuing. This registry is designed to evaluate the local factors that play a role in mediating or reflecting myocardial or coronary responses. Pathophysiologic processes of particular interest include restenosis, plaque ruptures, and angiogenesis. Study of the pericardial fluid bank should lead to enhanced understanding of molecular mechanisms, as well as to the explanation for the reasons underlying interpatient variability in these processes. It is further anticipated that this information might provide a foundation for the diagnostic use of pericardial fluid to individualize therapies targeting angiogenesis or plaque instability.

Key words: angiogenesis, cytokines, database, growth factors, pericardial fluid, plaque stability, registry

Introduction

Explanations for differences in coronary disease outcomes among patients may be found in specific local cellular and molecular events, which in turn might be reflected as well as modulated by the intrapericardial fluid which bathes the coronary vessels and myocardium. Although classically defined as an ultrafiltrate of plasma,1 pericardial fluid actually varies significantly from plasma with respect to its composition and concentration of a range of factors.2 3 This is so because it is locally conditioned by vascular tissues as well as myocardiocytes and mesothelium. Cytokines and growth factors present in the pericardial fluid microenvironment may thus relate to cellular events such as the activation of macrophages and endothelial cells in the coronary vasculature. Such activation, in turn, may affect expression of factors or enzymes resulting in angiogenesis or plaque instability.3

We have hypothesized that the pericardial fluid composition may be of potential significance for (1) understanding molecular mechanisms of these responses, (2) understanding the reasons underlying interpatient variability in these processes, and (3) providing diagnostic information to permit individualization of therapies targeting angiogenesis or plaque physiology. These hypotheses may be evaluated by analyzing specific components of pericardial fluid in relation to angiographic findings and clinical syndromes.

In initial studies, key factors thought to be important in angiogenesis and vessel wall growth control have been assessed. These include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, soluble vascular cell adhesion molecule (sVCAM), transforming growth factors (TGFs), monocyte chemotactic protein-1 (MCP-1), and macrophage colony-stimulating factor (M-CSF).

A pericardial fluid bank is a novel resource and is expected to provide new information concerning individual angiogenic capacity, plaque instability, and the local effects of the constellation of growth factors present in this environment.

Methods

The registry consists of samples and data of patients from participating centers who undergo cardiac procedures requiring pericardiotomy for either coronary bypass surgery or valve replacement. Patients are enrolled at the time of surgery and pericardial fluid is collected. As much fluid as possible is

Address for reprints:
Keith L. March, M.D., Ph.D., FACC
Krannert Institute of Cardiology
1111 West 10th Street
Indianapolis, IN 46202, USA
M-CSF. Other factors will be tested as suggested by experi-
mental results.

The associations among the molecular entities, clinical
t factors, and collateral vessel status are examined statistically
in several ways. Data undergo square root and logarithmic
transformations as needed to approach normal distributions.
Correlation coefficients, t-tests, and analysis of variance
(ANOVA) with Tukey's Honestly Significant Difference test
for multiple comparisons are used to examine specific rela-
tionships between clinical factors and molecular character-
istics. Logistic regression will be used to determine whether
a subset of cytokine measurements and clinical data can iden-
tify subjects who have the capacity to develop collaterals.

Some individuals have comparatively limited volumes of
pericardial fluid, which can limit the number of tests that can
be performed on any given sample. In such instances, analyses
are conducted to optimize the distribution of data among the
various entities being evaluated.

Results

To date, approximately 400 fluid samples have been ob-
tained from patients undergoing surgical pericardiotomy.
Volumes of fluid collected have ranged from 0.5 to 21.5 ml
(4.1 ± 0.34 ml, mean ± standard error of the mean). Reviews
of the chart-derived and angiographic data have been conducted
for these patients and assembled into a database as described
above. The present demographics of this population are shown
in Table I. Blood group percentages are illustrated in Table II.

To amplify the rate of recruitment of samples of pericardial
fluid and the associated data, this study has been extended to
include other centers.

Discussion

To date, comparatively little work has been devoted to
exploring the potential diagnostic utility of pericardial fluid. Its
use in this regard has been predominantly limited to the evalua-
tion of intrapericardial malignancy or infectious disease.
Only recently has pericardial fluid been considered as a fluid
to sample in the assessment of myocardial or coronary vascu-
lar processes. It has been recognized in several small studies
that this fluid contains a range of potent factors, with signifi-
cant interpatient variations in composition and concentration.

Elevated bFGF levels have been found in a study of 12 pa-
tients with unstable angina. In another study of 17 patients,
concentrations of bFGF in the pericardial fluid were found to
be as high as 20-fold that of serum, and the bFGF present was
found to contribute significantly to a trophic effect on adult
human cardiac myocytes. Furthermore, remarkable levels of
endothelin-1 have been identified in pericardial fluid, and
patients with heart failure have manifested elevated intraperi-
cardial levels of atrial natriuretic peptide (ANP) in an analysis
of 20 individuals. It is noteworthy that the levels of several
such factors are typically many times higher than the serum

<table>
<thead>
<tr>
<th>Table I</th>
<th>Demographics, coronary syndromes, and risk factors</th>
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<tbody>
<tr>
<td>Age</td>
<td>61.9 ± 11.8 years</td>
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<td>Gender</td>
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<tr>
<td>Hypercholesterolemia (%)</td>
<td>32</td>
</tr>
</tbody>
</table>

Abbreviations: CABG = coronary artery bypass grafting, CAD = coronary artery disease.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Blood group percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46%</td>
</tr>
<tr>
<td>B</td>
<td>9%</td>
</tr>
<tr>
<td>AB</td>
<td>3%</td>
</tr>
<tr>
<td>O</td>
<td>42%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Rh − = 16%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rh + = 84%</td>
</tr>
</tbody>
</table>

References:
levels. Representative pericardial:serum ratios include those for ANP, 90:1;8 brain natriuretic peptide (BNP), 20:1;8 endothelin, 36:1;6 and bFGF as above. These examples illustrate the fact that these cytokines and growth factors present in the pericardial fluid may play a significant role in mediating local physiologic processes and support the effort to expand the scope of studies on pericardial fluids, as will be facilitated by this pericardial fluid repository.

Conclusions

Pericardial fluid analysis may provide new prognostic information with respect to angiogenesis, plaque stability, or other cardiac diseases. It may also provide therapeutic direction with respect to specific needs in the areas of growth factor and other coronary management approaches. This fluid bank and database were designed to allow analysis of relationships among different clinical syndromes and different growth factors and cytokines.

The existing sample collection and database, although substantially larger than any similar collection currently available, will be rendered more powerful by continued expansion. In hopes to broaden this database in the future for further studies, all hospitals/research centers are invited to participate in this registry and to contact the investigators using the "Address for reprints."

References