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Nanotechnology in Cardiology

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ABSTRACT. The incidence of atherosclerosis, accompanying acute coronary syndromes and specifically, vulnerable plaque remain significant issues, both medically as well as a considerable cost to the healthcare system. With an estimated 180 million individuals affected at various stages of the disease process, clinically symptomatic disease accounts for approximately 34 million patients worldwide.

The need for improved therapies for cardiovascular diseases and the rationale for angioplasty and stent therapy is based on the underlying pathology of the disease process.

Recently, a new focus has emerged on an approach for the site-selective delivery of therapeutic agents to areas of the injured or dysfunctional vascular wall including vascular segments at risk

A number of important properties of these nanoparticles make them ideal as targeted delivery vehicles, including: 1. Increased adherence to damaged vasculature and endothelium; 2. Ability to non-covalently complex selected compounds; and 3. Potentiation of compound uptake by cells or tissue. The most well studied and the focus of the present report is the use of perfluorobutane / dextrose / albumin nanoparticles. Drugs can be incorporated into the microbubbles in a number of different ways, including binding of the drug to the microbubble shell and attachment of site-specific ligands. As perfluorocarbon-filled microbubbles are sufficiently stable for circulating in the vasculature as blood pool agents, they act as carriers of these agents until the site of interest is reached.

Despite important pharmacologic and interventional strategies to treat atherosclerotic vascular disease, it remains a serious clinical problem today. Intravenous microbubbles have been developed which can be used to detect where endothelial dysfunction exists, and which can be targeted to detect inflammatory and pro-thrombotic mediators on the plaque surface. These same microbubbles can then be used for site specific delivery of agents which inhibit plaque progression. These novel diagnostic and treatment strategies have the potential to significantly alter patient outcomes in atherosclerotic vascular disease. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Kew words: *nanotechnology, atherosclerosis, coronary artery disease.*

Introduction:

Transluminal coronary angioplasty (percutaneous transluminal coronary angioplasty; PTCA) was introduced in the late 1970's as a nonsurgical treatment for obstructive coronary artery disease and blockage due to myocardial infarction. The procedure involves placing a balloon-tipped catheter at the site of occlusion and disrupting and expanding the occluded vessel by inflating the balloon. Although initially successful at removal of the blockage and luminal enlargement, the process also damages the blood vessel wall extensively including the loss of the endothelial lining. An ensuing response to this severe injury is often enhanced expression of cytokines and growth factors, and subsequently, a rapid acute re-closure and/or a slow progressive re-occlusion or restenosis of the vessel. Within the vascular wall, this response typically includes myointimal hyperplasia, proliferation of smooth muscle cells and

fibroblasts, connective tissue matrix remodeling, and formation of thrombus. Restenosis, referring to the re-narrowing of the vascular lumen following an intervention such as balloon angioplasty, is clinically defined as a greater than 50% loss of the initial luminal diameter gain following the interventional procedure and has affected anywhere from 25 – 35% of treated patients [1,2].

Today, standard therapy for myocardial infarction or other luminal narrowing includes thrombolytics, anticoagulants, and often, interventional procedures such as PTCA. Recently, an advance to the procedure has been the introduction of stents, metallic-based cage/tube-like structures placed into the vessel lumen with PTCA, and the rate of acute reclosure has been minimized. Coronary stents provide luminal scaffolding, eliminating elastic recoil and remodeling which can occur rapidly following an interventional procedure. Unfortunately however, although the occurrence of acute reclosure was reduced, there was actually no decrease in neointimal hyperplasia, and in fact, the procedure led to an increase in the proliferative component of restenosis and a relatively higher rate of reocclusion and need for re-intervention often as early as at 3 months to 1 year following the procedure [3].

Recently, increased success has been achieved against the PTCA associated neointimal hyperplasia through the invention and development of drug eluting stents (DES) [4,5]. Drug coated or impregnated stents deployed within the lumen of the blood vessel have been developed where a given drug is gradually (days to weeks) eluted, diffusing into the proximal vessel wall [6]. Examples of compounds used include inhibitors of mTOR such as rapamycin (Sirolimus.RTM, Wyeth Ayerst), a macrolide immunosuppressive agent, as well as chemotherapeutic such as paclitaxel (Taxol., Bristol-Myers Squibb) or actinomycin D. With the recent development of angioplasty combined with DES such as the Cypher®-Coronary Stent marketed by Cordis / J&J Pharmaceuticals, treatment of the culprit vessel in myocardial infarction has had a significant advance. Rapamycin (sirolimus), the agent utilized in this first successful DES is an immune mediator shown to quiet the local immune activation, and also, to reduce or eliminate cellular proliferation. Boston Scientific, with their Taxus / paclitaxol DES, has also shown success. All of these compounds have been shown to inhibit smooth muscle cell proliferation [7-10], and have reduced the rate of restenosis to the 6 – 10% range [11-15]. Locally, the drug eluting stents have been shown to be very effective on the treated lesion, effectively reducing the restenotic process and maintaining patency of the treated vessel over the long term. Their use has changed the paradigm for interventional cardiology and has become the standard of practice in most developed countries.

Despite the advances of DES, a number of critical issues remain in the treatment of cardiovascular disease and the current focus on plaques with the largest stenosis. These concerns include not only the cost to the health care systems, but far more importantly, true overall benefit to the patient with respect to more serious cardiovascular events. Recent evidence suggests that although treatment of the largest lesion is now successful with PTCA and DES, and that the overall event rate including specifically the need for re-intervention within the treated vessel is reduced, the more serious events such as a second MI have not changed dramatically [16]. In addressing this issue, it has recently been documented in patients undergoing PTCA due to an event with plaque rupture that there was evidence of additional ruptures at sites distal to the culprit or stented lesion. Importantly, it is plaque rupture and exposure and release of underlying procoagulant/thrombotic components that appears to be responsible for most acute cardiovascular events. By utilizing intravascular ultrasound (IVUS) in patients undergoing angioplasty for an infarcted artery, Rioufol et al [17] (2002), observed distal rupture sites in at least 80% of patients examined. These ruptures typically occurred in plaques that were less than 50% stenosed, and thus their detection likely would have been overlooked by angiography. This finding suggests that treating the culprit lesion alone as is accomplished with stent therapy is not sufficient, and that intervention at multiple active lesion sites will be required to reduce secondary events and mortality.

With respect to costs, approximately 2 million PTCA/stent procedures are performed per year today worldwide, with an estimated cost of over $5 billion for the stents alone (> $3000/ stented patient). Unfortunately, the expense of DES is an issue of increasing concern, essentially tripling that of bare metal stents. With multiple stents often implanted into individual patents, this change in clinical practice has had a significant impact on health care costs.

Finally, in addition to the issues described above for costs and secondary events, treatment is also lacking for many more at-risk patients who cannot undergo successful angioplasty with stents. These patients, who may have either diffuse, non-stentable lesions, bifurcated lesions, multi-vessel disease (i.e. diabetics), or those who have failed primary angioplasty with stents, are not benefiting as much from DES, and improved treatments here also remain a clear clinical need.

Needs Beyond PTCA and Restenosis: Cardiovascular Disease and Vulnerable Plaque

The incidence of atherosclerosis, accompanying acute coronary syndromes and specifically, vulnerable plaque remain significant issues, both medically as well as a considerable cost to the healthcare system. With an estimated 180 million individuals affected at various stages of the disease process, clinically symptomatic disease accounts for approximately 34 million patients worldwide [18]. Nearly 2600 Americans die of cardiovascular disease every day, an average of one death every 34 seconds. This accounted for 38.5% of all deaths or one of every 2.6 deaths in the United States in 2001. Additionally, according to the NHLBI, the cost to the United States in 2004 was $368 billion for total cardiovascular disease care. When divided between the interrelated cardiovascular diseases (stroke, coronary artery disease, hypertension and congestive heart failure), costs for coronary atherosclerosis specifically is the largest.

The need for improved therapies for cardiovascular diseases and the rationale for PTCA therapy is based on the underlying pathology of the disease process. Atherosclerosis has been described as a chronic inflammatory syndrome, a systemic disorder typified by focal lesions throughout the vasculature [19,20]. In the past, plaques have been regarded as being inert and remaining almost unchanged for years. However, work over the past two decades demonstrates that plaques are very active entities and vulnerable plaques, those with the propensity to crack or rupture and initiate vascular events, are sites of intense inflammatory activity [21,22].

One of the initial signs of atherosclerotic disease is endothelial dysfunction, characterized as such due to an inappropriate constrictive response to normally vasodilatory agents (e.g. acetylcholine) [23-25]. Additionally, within the focal lesions of atherosclerotic plaque are sites of increased metabolic activity. These regions differ from non-diseased areas and are characterized by the presence and clustering of immune cells such as macrophages and T-cells along with corresponding expression of signaling molecules such as cytokines, chemokines, and degradative enzymes [26-28]. These disease fighting components are critical for overall health, but locally, strongly contribute to and direct the atherosclerotic process. Inflammation is a key factor in all 3 phases of the atherosclerotic process, which can be divided into 3 phases: 1) lesion initiation (atherogenesis), 2) lesion progression, and 3) rupture and thrombosis. Specifically, it is through the activities of these immune mediators that rupture of the surface of the lesion occurs leading to occlusion and cardiovascular events [29-31]. Thus, within the metabolically distinct regions of vascular plaque, targeted local regulation of the activities of these mediators holds considerable promise for future treatments.

It has been recognized recently that rupture and infarctions occur in vessels with plaques that are only mild to moderately obstructed, more often than not, in vessels less than 50% stenotic on angiography [32-34]. The concept of vulnerable plaque, characterized by a large lipid core with a high content of inflammatory cells and a thin fibrous cap, has received considerable attention [35-37]. These lesions, typically only mildly stenotic, can rupture, and are responsible for most acute coronary thrombosis leading to myocardial infarction. Often, in vulnerable patients, there is a large systemic plaque burden with multiple focal regions of vulnerable plaque, and thus, a need for therapy at multiple sites simultaneously. Mortality here remains high, and short of death, rupture of plaques is associated with significant morbidities including stable and unstable angina as well as NSTEMI and STEMI [38,39]. Consequently, vulnerable plaques and vulnerable patients, those having a high systemic total plaque burden, remain of substantial concern.

Based on this information, there is clearly a need for new, targeted, therapies to quiet the local inflammation within the specific areas of disease of the vascular wall, not solely the largest areas of occlusion as is addressed with PTCA and DES. Such therapy would be of importance for secondary intervention following an initial event as described above, where there is documentation of multiple sites of rupture, for patients with non-stentable, diffuse or multi-vessel disease, and potentially for use as primary prevention in those patients with documented atherosclerotic disease and elevated immune markers.

Nanoparticle Vehicles

Recently, a new focus has emerged on an approach for the site-selective delivery of therapeutic agents to areas of the injured or dysfunctional vascular wall including vascular segments at risk for restenosis following percutaneous coronary interventions. The technology is not a stent or device approach, but rather a single i.v. infusion that allows for a targeted delivery of compounds to sites of vascular dysfunction or injury. Components of the technology include a combination of known / approved ultrasound contrast vascular imaging agents complexed with therapeutic agents. Ultrasound

contrast agents have been used in diagnostic echocardiology for several decades, and use of such agents has been investigated for the transport and delivery of therapeutic agents, and is well reviewed by Bekeredjian [40].

A number of important properties of these nanoparticles make them ideal as targeted delivery vehicles, including: 1. Increased adherence to damaged vasculature and endothelium; 2. Ability to non-covalently complex selected compounds; and 3. Potentiation of compound uptake by cells or tissue. The most well studied and the focus of the present report is the use of perfluorobutane /dextrose/ albumin nanoparticles. Drugs can be incorporated into the microbubbles in a number of different ways, including binding of the drug to the microbubble shell and attachment of site-specific ligands. As perfluorocarbon-filled microbubbles are sufficiently stable for circulating in the vasculature as blood pool agents, they act as carriers of these agents until the site of interest is reached.

Albumin/dextran /perfluorobutane gas microcarriers (PGMC)s:

Albmin-coated gas microbubbles do not adhere to normally functioning endothelium. However, adherence does increase considerably to activated or dysfunctional endothelial cells or to extra-cellular matrix of the disrupted vascular wall, an interaction that could be a marker of endothelial integrity [41]. A second interesting feature of these nanoparticles is the ability to non-covalently complex selected compounds to them, thus allowing for the concentration of the compounds on the particles, and potential for transport with them. Thirdly, these nanoparticles have been demonstrated to be taken up by cells of importance to the diseased vasculature, and, also through a cell membrane fluidizing effect, to enhance the transit of compounds to these cells. Thus, theoretically, delivery of drugs or genes bound to albumin-coated microbubbles could be selectively delivered and concentrated at sites of most therapeutic need [42,43].

Recent evidence suggests that PGMCs (nano-particles) can be utilized as local delivery vehicles for compounds to regions of injured or diseased vasculature. These particles are routinely used in Europe, South America and Asia as ultrasound contrast agents in patients, and have been studied extensively. They are prepared as a liquid suspension of microbubbles containing a blood-insoluble gas by mixing 5% human serum albumin and 5% dextrose with decafluorobutane and briefly sonicated to a consistent size. Once formed, the PGMC are between 0.1 and 10 μm in diameter, are nontoxic, and are gaseous at body temperature with diffusion coefficient and blood solubility lower than oxygen or nitrogen. Extensive documentation supports the concept that when injected i.v., these circulating nano-particles tend to adhere and are retained preferentially to the denuded or dysfunctional luminal surface of the vascular wall [44-46].

Mechanistically, studies support a role for both the endothelium and for leukocytes in nanoparticle retention. In vitro, images obtained on light microscopy illustrate nanoparticle attachment to the surface of activated neutrophils at 3 minutes and phagocytosis of microbubbles by 15 minutes [47]. At 30 minutes, the bubbles were no longer apparent. Villanueva reported that during pathophysiologic states associated with endothelial dysfunction, microbubbles adhere to disrupted vascular endothelium and that this interaction can be used as a marker of endothelial integrity. In a series of studies exploring retention by cultured endothelial cells (ECs), they have found that the particles do not adhere to normal confluent ECs. However, upon immune activation with phorbol ester, there was enhanced adherence to the cells, and especially to the extracellular matrix produced and exposed under inflammatory conditions. Within the context of vascular disease, nano-particle adherence to the dysfunctional endothelium occurs mainly due to destruction of the negatively charged glycocalyx protecting the endothelium and binding of microbubbles to activated leukocytes slowly rolling over the damaged endothelial surface [48].

Numerous in vivo studies also support selective adherence of microbubbles to damaged or dysfunctional vasculature. Linder found in vivo that microbubbles quickly attach to activated leukocytes that are adherent to the endothelium after ischemia-reperfusion injury and also, during tumor necrosis factor-α (TNF-α)–induced inflammation in the mouse [49]. As studied by intravital microscopy, nano-particle interactions with the few adherent leukocytes were uncommon when observations were made both early (0 to 2 minutes) and late (15 to 17 minutes) after their intravenous infusion in control mice. In contrast, treatment with TNF-α resulted in a far greater number of microbubble (nano-particle) interactions with the abundant adherent leukocytes at both time points. Microbubbles attached to the leukocyte surface early after injection, and most appeared to be phagocytosed by 15 to 17 minutes, at which time freely circulating microbubbles were only occasionally observed. This attachment was ascribed to their $ß_2$-integrin– and complement-mediated binding to activated leukocytes adherent to the vascular wall.

In addition, as shown by Lindner [50] using ultrasound imaging, the distribution of PGMCs was affected in models of vascular dysfunction or injury, including the hyperlipidemic and injured pig. In the setting of endothelial dysfunction (ED) induced by hyperlipidemia, Tsutsui [51] found in the pig that retention of intravenously injected albumin microbubbles occurs in the setting of both global and regional ED in large vessels. Microbubbles normally pass freely through large and small vessels but are retained in regions with ED. Intravenous albumin-encapsulated microbubbles were administered in seven pigs while imaging the carotid arteries before and after a 20% intralipid infusion to induce hypertriglyceridemia. The degree of microbubble retention was quantified by measuring endothelial acoustic intensity (AI) by ultrasound after clearance of free-flowing microbubbles, and the arterial diameter responses to acetylcholine were quantified. After induction of hypertriglyceridemia, adherence of the microbubbles was visually evident in all carotid arteries, and endothelial AI increased significantly ($p < 0.001$ compared with baseline). The arterial responses to acetylcholine went from vasodilation at baseline to vasoconstriction during hypertriglyceridemia. Endothelial AI also increased in balloon-stretched vessels ($p < 0.01$ compared with uninjured vessels) after albumin-encapsulated microbubble injection, with a ring of microbubbles selectively adhering to the injured segment, and scanning electron microscopy confirmed that albumin-coated microbubbles adhered to endothelial cells.

Finally, in a study recently completed exploring the mechanism of adherence, fluorescein-labelled PGMC (PGMC-FITC) nanoparticles were prepared and infused into C57BL/6J mice 24 hrs. after wire induced aortic endothelial injury (Porter TR et.al. 2006: Personal Communication). PGMC-FITC microbubbles were attached to the endothelium of injured aorta mice. The number of microbubbles in the different fields was variable, reflecting the heterogeneous pattern of injury caused by the wire. In the non-injured mice, it was reported that there were only isolated PGMC nanoparticles adherent to the aortic injured endothelium. By quantitative analysis, the number of microbubbles in the injured aorta (31 ± 40 bubbles/field) was significantly higher than in non-injured mice where only rare bubbles were observed in the proximal non-injured aorta (2 ± 4 bubbles/field; $p < 0.0001$.

Importantly, only rare bubbles were observed in the proximal non-injured aorta, both in the non CVF-treated (2 ± 4 bubbles/field; $p < 0.001$ versus injured aorta) and in the CVF-treated mice (0 ± 1 bubbles/field; $p < 0.001$ versus injured aorta). Taken together, there is substantial evidence supporting the concept that albumin-containing microbubbles (nanoparticles) can target selectively and be retained to regions of importance to vascular disorders. These regions are characterized by dysfunctional or denuded endothelium where appropriate therapy will have a significant impact on reduction of events. Because these adhered microbubbles retain their acoustic reflectivity, they can be visualized with specialized low mechanical index ultrasound transducers. These transducers have been shown to detect retained albumin microbubbles in the setting of endothelial dysfunction produced by either balloon injury (or hypertriglyceridemia.

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Drugs for Delivery

A number of therapeutic agents have been explored for incorporation into the nanoparticle delivery vehicle and for efficacy on restenosis. Among the agents chosen are two which have previously been demonstrated to be effective on vascular disease including within the DES paradigm (sirolimus, paclitaxol), and a third, antisense to c-myc, with a promising mechanism of action. All three have significant issues (toxicity, stability) upon systemic administration. By combining with the nanoparticle delivery system, with the potential for targeted delivery of these agents to the local site of vascular injury, concerns of systemic liabilities will be minimized. If successful, it is anticipated that this approach will be of benefit to PTCA, and is reasonable to assume that it can be used in combination with stents when appropriate, both DES and bare metal. Additionally, the technology has potential for use well beyond PTCA and includes additional indications such as ACS, PAD, vulnerable plaque and atherosclerosis.

The feasibility of nanoparticle mediated site selective delivery of compounds to injured or dysfunctional

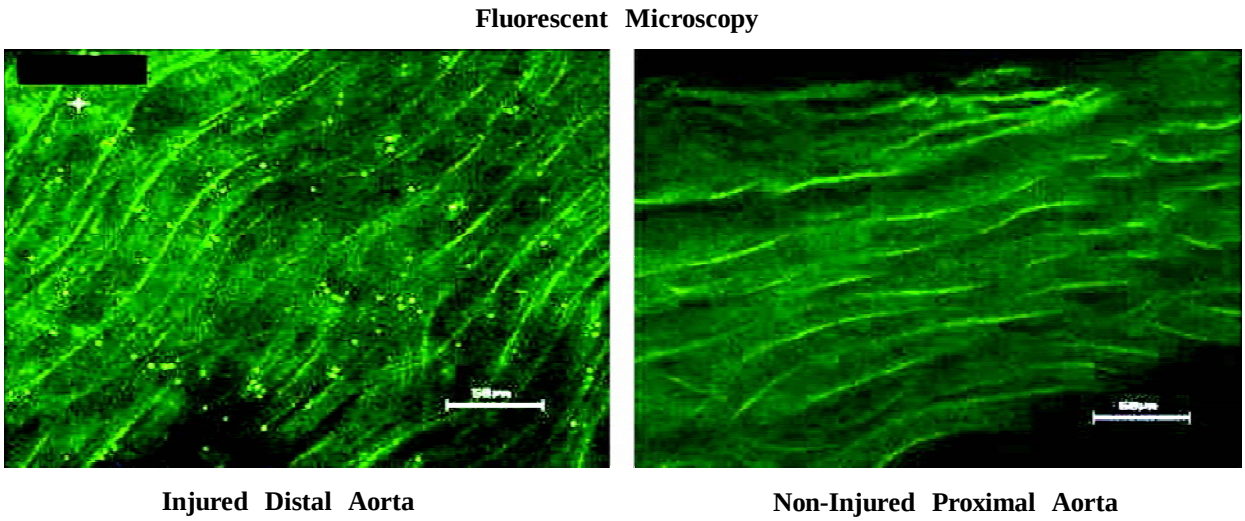


Fig. 1. FITC-labeled PESDA microbubbles adhere selectively to the site of mouse aortic injury (left panel). This was not seen in the non-injured proximal aorta of the same mouse (middle panel) or in the injured aorta of a mouse treated with cobra venom factor (right panel) to deplete complement. Scale bar = 50 microns.

regions of the vascular wall has been demonstrated. Recent studies demonstrate that bioactive compounds (i.e. genes, antisense, protein, etc. as well as small molecules such as sirolimus or paclitaxol can be incorporated into nanoparticles and, with the particles acting as targeting vehicles, be delivered preferentially to sites of vascular injury. As summarized by **Feinstein** [52] and Bekeredjian [53] preparations of compounds at relatively high concentrations have been incorporated into these nanoparticles using simple and straightforward techniques such as sonication. In addition, as discussed below, studies focused on the delivery of antisense to c-myc or sirolimus undertaken by Kipshidze [54,55] Kipshidze NN, Porter, TR, Dangas G et al., systemic targeted delivery of antisense with perflourobutane gas microbubble carrier reduced neointimal formation in the porcine coronary restenosis model [54] in pigs, or paclitaxol [56] in rabbits further validate the potential showing that systemic delivery targeted with perflourobutane gas microbubble carrier reduced neointimal formation in the porcine coronary restenosis model.

Rapamycin (Sirolimus)

Rapamycin is a macrolide antibiotic approved for use as an immunosuppressive agent in the prevention of organ rejection following renal transplantation. Acting through the nuclear cell cycle regulator TOR, the compound has been found to have pleotropic effects on cellular metabolism and is a novel inhibitor of growth factor & cytokine-stimulated cell proliferation. Numerous studies have found this compound to be protective against vascular disorders, both in animal models as well as in patients. As summarized by Marks [57], sirolimus has a number of effects of consequence on the processes of restenosis and vascular disease at the molecular level including the inflammatory response and the proliferative and migratory activity of smooth muscle cells [58]. In vivo, studies have demonstrated efficacy of rapamycin on vascular disease from a diverse mix of animal models thought to appropriately mimic aspects of human vascular disorders. Initially, Gregory et al [59] demonstrated that rapamycin was a potent inhibitor of the intimal thickening which occurs following balloon injury of the carotid artery in the rat. Subsequent studies by Gallo et al [60] reported that rapamycin significantly reduced the arterial proliferative response after PTCA in the pig. These studies demonstrating efficacy on induced vascular injury ultimately led to the development of the Cypher®-stent, the first drug (rapamycin) eluting coronary stent. In addition to induced injury models, Elloso [61] and then Basso [62] and Waksman [63] demonstrated inhibitory effects in the apo E deficient mouse model of atherosclerosis by sirolimus on the development and morphology of the plaque, and then, on reduced vascular cholesterol content, and in some of these studies, despite an enormous circulating systemic cholesterol load (up to 1300 mg/dl).

In the Clinic, the success of the Cypher-DES for the prevention of restenosis following angioplasty has been well documented [4,5]. By engineering the device to elute rapamycin over a 14 day period [6], intimal thickening and restenosis formally associated with angioplasty is now reduced to approximately 6% over the long-term, and utilization of these drug-eluting devices has brought

about a new era in the practice of interventional cardiology. More recently, studies of rapamycin by Ikonen [64] in non-human primates have shown lesion inhibition and possibly regression in vascular allograft rejection models, demonstrating efficacy in a severe immune mediated vascular situation. Finally, clinical studies by Mancini [65] and Eisen [66] on vasculopathy and also Keogh [67] on coronary artery disease undertaken in subjects who have undergone heart transplantation have demonstrated that rapamycin (or analogs) has the ability to maintain patency and potentially reverse stenosis of coronary vessels. Based on the above, sirolimus would appear to be an ideal candidate as a therapeutic agent for cardiovascular diseases. However, based on its immunosuppressive activity and systemic toxicities, an improved and targeted approach for delivery is required to take full advantage of its potential benefit.

In studies utilizing nanoparticle (PGMC) based technology in pigs undergoing balloon / stented angioplasty, it has been demonstrated that selective delivery of sirolimus to an injured region of the coronary vasculature could be achieved [68]. In the study by Kipshidze et al, following a single i.v. administration, blood concentrations of sirolimus peaked at 10 minutes and were below the limit of detection after 24 hours. Analysis of balloon injured coronary vessels showed that sirolimus delivered via PGMCs significantly up-regulates expression of p27 by Western blots in injured areas, a marker of sirolimus biologic activity. Tissue drug concentration from injured arteries showed total sirolimus delivered to the vessel wall at 4 hours was from 169.6 to 240.8 ng per vessel site [note: vessel mass ~40 mg]. Drug was homogeneously distributed in the stented areas and in areas adjacent to the stent. Critical to the approach, there was no drug detected in the remote vasculature such as intact coronary or carotid arteries (limit of detection = 0.01 ug/g tissue). Additionally, no drug was detected in injured vessel regions after 72 hrs.

As shown below, at 28 days following angioplasty and sirolimus/nanoparticle infusion, there was a 40% reduction in neointimal area in treated animals. Control arteries exhibited a substantial neointima consisting mostly of satellite and spindle-shaped cells in a loose extracellular matrix. Histopathoxlogy showed no difference in endothelialization score, SMC content, inflammation or fibrin deposition between groups. No toxicity or evidence of myocardial damage was seen on gross inspection or after histological examination of the heart. No thrombosis of treated segments was observed.

These studies demonstrated that in the porcine coronary model, site specific systemic delivery of Rapamycin using PGMC resulted in: (i) high target-vessel tissue concentration of rapamycin, (ii) enhanced local expression of p27, and (iii) reduced neointimal formation by 40% at the target site 28 days post procedure. All these effects provide solid basis for further preclinical investigation of this novel mode of therapy.

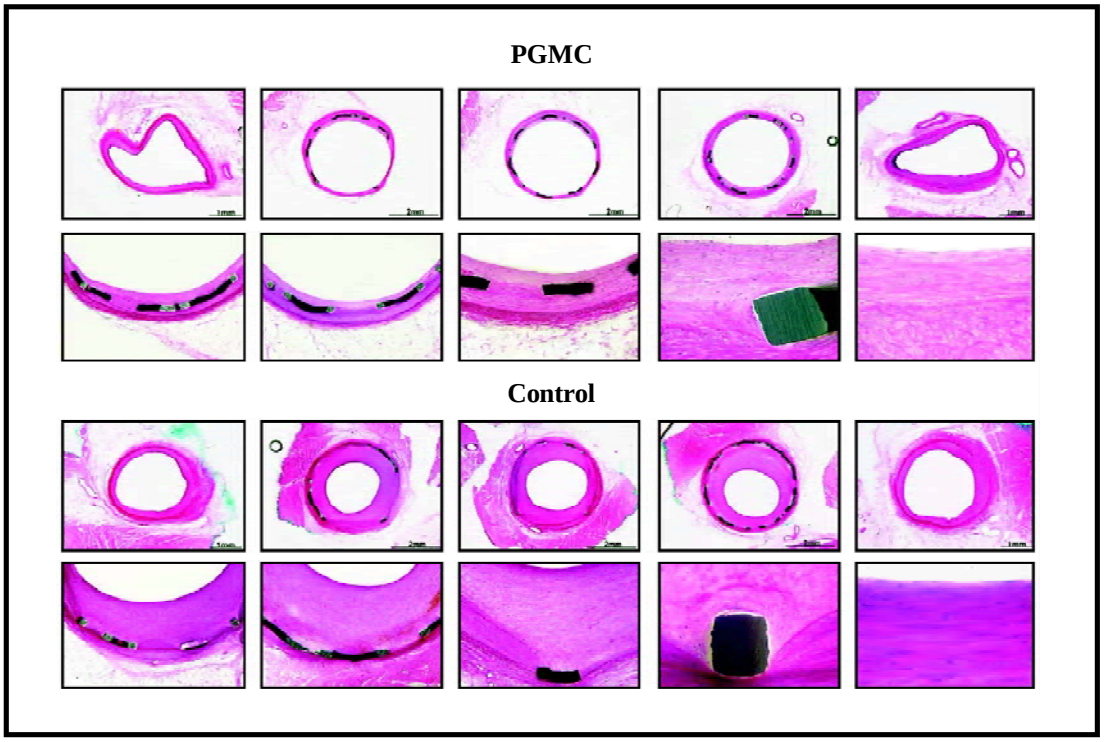
Antisense Therapy: antisense for c-myc AVI-4126

The recent advances in gene therapy and molecular biology have improved the interest in methods of noninvasive or non-systemic delivery of therapeutic agents. However, the clinical applicability of antisense technology has been limited due to a relative lack of target specificity, slow uptake across the cell membranes, and rapid intracellular degradation of the oligonucleotide. It has already been demonstrated that perfluorocarbon-filled albumin nanoparticles avidly bind proteins and synthetic oligonucleotides [69-71]. In a similar way, these particles can directly take up genetic material, such as plasmids and adenovirus, suggesting a potential for protection of the material and targeted delivery via nanoparticles.

As discussed, restenosis after vascular balloon injury or stent deployment has been shown to result from neointimal hyperplasia due to smooth muscle cell migration and proliferation. The c-myc protooncogene is responsible for the regulation of gene expression involved in the process of intimal hyperplasia that leads to restenosis. Synthetic antisense oligonucleotides, such as those to the c-myc protooncogene, can bind to the messenger ribonucleic acid and inhibit the synthesis of the protooncogenes. Therefore, antisense to c-myc protooncogene can prevent its translation into proteins that may be mediators of the pathologic process of restenosis. In testing this hypothesis, the phosphorodiamidate morpholino oligomer antisense to c-myc, AVI-4126 was bound to perfluorobutane gas microbubble carriers and injected systemically into pigs and assessed for activity on expression of c-myc in vascular tissue and restenosis after stent implantation.

The effect of the nanoparticle-based systemic delivery of antisense to c-myc on neointimal hyperplasia after 28 days in a swine stent restenosis model was investigated. HPLC analysis of plasma samples of treated animals showed minimal presence of AVI- 4126. However, analysis (HPLC) of stented vessel tissue showed delivery of AVI-4126 at 4 hours. Western blot analysis demonstrated that stent implantation lead to activation of c-myc oncogene. However, when an AVI-4126 loaded

stent was implanted there was significant inhibition of c-myc expression by Western blot analysis of the vessel tissue Morphometry showed that the neointimal area was significantly reduced in the AVI-4126: PGMC group compared with control (2.63± 1.99 vs. 4.77±.1.71 mm2 respectively, $p<0.05$). The quantitative histomorphometry demonstrated a statistically significant reduction of IA-value, and IA normalized to injury score (IA/IS) following treatment. There was also a significant difference in the lumen area between the antisense treated compared to controls, thus, similar to the sirolimus delivery via nanoparticles, systemic targeted delivery of AVI-4126



Data at 28 days	Control	Rapamycin	P value
Vessel Area (mm^2)	9.74 ± 1.26	10.02. ± 2.17	NS
Lumen Area (mm^2)	3.34 ± 0.72	6.55 ± 2.69	<0.05
Intimal thickness (mm)	0.583 ± 0.207	0.335 ± 0.161	<0.05
IA (mm^2)	4.77 ± 1.71	1.84 ± 0.84	<0.001
Media Area (mm^2)	1.60 ± 0.24	1.62 ± 0.46	NS
Area % Occl.	57.53 ± 13.19	26.13 ± 19.00	< 0.05
IS	1.92 ± 0.63	1.75 ± 0.46	NS
IA/IS Ratio	2.48+-0.67	1.05+-0.39	<0.01
Inflammation Score	0.67 ± 0.52	0.44 ± 0.13	NS
Intimal Vascularity	0.42 ± 0.52	0.38 ± 0.48	NS
Intimal Fibrin	0.17 ± 0.14	0.19 ± 0.24	NS
Intimal SMC Content	3.00 ± 0.00	3.00 ± 0.00	NS
Adventitial Fibrosis	1.17 ± 0.76	0.88 ± 0.25	NS

using PGMC carrier significantly inhibited neointimal formation of the porcine coronary stent model,

Paclitaxel

Paclitaxel, a potent antineoplastic drug, interferes with the assembly of microtubules and thus interferes with cell migration and replication. It has been shown to be effective on mechanisms of restenosis, but as a chemotherapeutic agent with significant systemic toxicities, it is best administered locally for cardiovascular uses. Success has been achieved with local delivery on a drug-eluting stent which allows for achievement of therapeutic concentrations without the risk of systemic toxicity [72]. However, paclitaxel eluting stents in animals cause incomplete healing and, in some instances, a lack of sustained suppression of neointimal growth [73].

The efficacy of paclitaxel as delivered on an albumin-stabilized systemic delivery nanoparticle for reducing in-stent restenosis was tested in rabbits [56]. In New Zealand White rabbits receiving bilateral iliac artery stents paclitaxel was administered as a 10-minute intra-arterial infusion. Pharmacokinetics showed a biphasic profile with an initial rapid decline in concentration followed by a slower elimination phase. Whole blood concentrations were maximal at 15 minutes after infusion and ranged from 3.0 to 5.0 μmol/L. At 24 and 48 hours, blood levels were approximately 0.1 μmol/L. The total arterial tissue level of paclitaxel in the stented segments at 48 hours was 1.7±0.1 μg/mg of tissue. At 28 days, mean neointimal thickness was reduced, with evidence of delayed healing. The efficacy of a single dose, however, was lost by 90 days. In contrast, a second repeat dose given 28 days after stenting resulted in sustained suppression of neointimal thickness at 90 days and nearly complete neointimal healing. Thus, this formulation of paclitaxel may allow adjustment of dose at the stent treatment site and prove to be a useful adjunct for the clinical prevention of in-stent restenosis.

Clinical experience

The aim of this study was to evaluate the efficacy and safety of a microbubble delivery of *c-myc* antisense RESTEN-MP^{TM} (AVI BioPharma supplied by Global Therapeutics LLC) in preventing restenosis after coronary stenting in *de novo* lesions of coronary arteries.

A MULTI-LINK ZETA bare metal stent was implanted in a de novo coronary artery lesion (RD 2.5mm and 4.0mm: TL 10mm and 30mm in length). Serial (baseline and 6-mouth follow-up) intravascular ultrasound analysis was performed in 20 lesions. A dose of 16mg RESTEN-MP^{TM} was administrated intravenously upon confirmation of stents implantation and again at 24 hours post stent.

34 out of 52 patients were enrolled at the study site. After stenting, the MLD and IVUS lumen volume was determined, 13 Patients had a LAD lesion., 14 LCX, 7 RCA. Approximately 2/3rds of the lesions were either Type B2 or C lesion. At six month follow-up, both the MLD and IVUS lumens were determined. Of the 20 patients currently studied at six-month in the IVUS substudy, only one TLR was required. Of particular interest were 2 cases in which the study stent remained patent and a previously implanted DES (Paclitaxel) showed a severe in-stent stenosis.

In summary of human study Microbubble delivery of *c-myc* antisense is effective in reducing neointimal tissue proliferation comparable to DES without the problem of late stent thrombosis due to a lack of complete re-endotheliazation.

Conclusions:

Despite important pharmacologic and interventional strategies to treat atherosclerotic vascular disease, it remains a serious clinical problem today. Intravenous microbubbles have been developed which can be used to detect where endothelial dysfunction exists, and which can be targeted to detect inflammatory and pro-thrombotic mediators on the plaque surface. These same microbubbles can then be used for site specific delivery of agents which inhibit plaque progression. These novel diagnostic and treatment strategies have the potential to significantly alter patient outcomes in atherosclerotic vascular disease.

სამედიცინო მეცნიერებანი

ნანოტექნოლოგიები კარდიოლოგიაში

ნ. ყიფშიძე

ნ. ყიფშიძის სახელობის ცენტრალური საუნივერსიტეტო კლინიკა, თბილისი, საქართველო, ლენოქს ჰილის ჰოსპიტალი, ნიუ იორკი, აშშ

სისხლძარღვთა ათეროსკლეროზული დაავადებების მკურნალობაში მნიშვნელოვანი მიღწევების, ფარმაკოლოგიური და ინტერვენციული სტრატეგიის მიუხედავად, ის დღეისათვის მაინც სერიოზულ პრობლემად რჩება. ბოლო დროს ყურადღება გამახვილებულია თერაპიული აგენტების სელექციურ მიწოდებაზე სისხლძარღვის კედლის დაზიანების ან დისფუნქციის არეებში.

ნანონაწილაკებს გააჩნიათ მთელი რიგი თვისებებისა, რომელიც მათ იდეალურს ხდის მიზანმიმართული მიწოდების თვალსაზრისით: 1. დაზიანებულ სისხლძარღვსა და ენდოთელიუმთან შეკავშირების გაზრდილი ხარისხი; 2. არაკოვალენტური კომპლექსის შესაძლებლობა; 3. უჯრედებისა და ქსოვილების მიერ ნივთიერებების შეთვისების პოტენცირება.

წარმოდგენილ შრომაში ყურადღება გამახვილებულია პერფლუორობუტან ალბუმინის ნაწილაკების გამოყენებაზე. წამლები შეიძლება ინკორპორირებულ იქნან მიკრობუშტუკებში სხვადასხვა გზით — მათ გარსთან წამლების მიბმით და განსხვავებული მდებარეობის ლიგანდების დაკავშირებით. რამდენადაც პერფლუროკარბონით გავსერებული მიკრობუშტუკები გაცლებით სტაბილური არიან სისხლძარღვთა სისტემაში სისხლის გადამტანი აგენტების ცირკულაციისათვის, ისინი მოქმედებენ როგორც ამ აგენტების მატარებელნი დასახული მიზნის მიღწევამდე.

ნანონაწილაკები შეიძლება გამოყენებულ იქნას ენდოთელიური დისფუნქციის და, ასევე, ანთებითი და პროთრომბული მედიატორების აღმოსაჩენად ფოლაქების ზედაპირზე. იმავე მიკრობუშტუკების გამოყენება შეიძლება განსხვავებული მდებარეობის აგენტებისათვის ფოლაქების პროგრესირების საინჰიბიციოდ. ამ ახალ დიაგნოსტიკურ და სამკურნალო სტრატეგიას შეუძლია მნიშვნელოვნად გააუმჯობესოს პაციენტის მდგომარეობა სისხლძარღვთა ათეროსკლეროზული დაავადებების დროს.

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