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USE OF CONJUGATE BIOTECHNOLOGY TO IMPROVE THERAPEUTIC OPTIONS

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For all therapeutic options local drug delivery should in theory be the preferred way of ensuring adequate drug is delivered to the pathological site without the risks of side effects. It would appear illogical to administer a drug systemically to treat perhaps only 20 to 30 mm of the coronary artery. Large systemic doses may be required to achieve adequate doses of drug locally, with the risk of systemic side effects. Sometimes agents shown to be effective in systemic doses in an animal model cannot be given to man in the same dose or are shown in clinical trials as a result to be ineffective. A good example was the lack of efficacy of ACE inhibitors in human trials of angioplasty restenosis despite their proven value in the rat and rabbit models: the dose effective in the model was 10 times that which could be tolerated by man. It would have been better to have calculated the dose required locally and to have delivered that locally. Local drug delivery can be achieved using appropriately designed balloons. A number of these and a number of agents have been extensively investigated in vitro and in animal studies. There are three major problems with local drug delivery. First, it is not local; many studies have shown that regional distribution of an agent occurs. Thus drug delivered to the coronary arteries could affect myocardial cell function. Second, efficiency of delivery is poor, with only 2% to 5% of drug being delivered to the site. Part of this is loss of drug from the device and part is lack of retention. While it is always difficult to determine how much (or how little) drug is needed locally, such studies do at least suggest that the majority of drug (> 95%) is not locally retained and is therefore in the systemic circulation. The final major problem is the lack of retention itself. There appears no reason, except for the passive adsorption of antibodies to smooth muscle cells for example, for the drug to stay where it is delivered.

In our laboratory we have, over a number of years, explored the possibility of improving retention and therefore efficacy of drugs delivered locally by the development of conjugates. Our initial work developed the concept of targeting through the use of localizing agents. Thus a conjugate would in effect be the combination of a localizing agent, usually an antibody directed against a particular component of the vessel wall, that was expressed by the pathological process, combined through a chemical process to the effector agent, a lower than systemic dose of the active drug. Initial studies assessed an antibody against damaged vessel wall (P14G11) conjugated using SPDP to a glycoprotein IIb/IIIa receptor blocker. Delivering this conjugate with an LDD balloon not only reduced restenosis in an in vivo model of angioplasty at a dose seven times less than would have been required systemically, but measures of systemic effects were not seen.

Since this early work we have been studying the development of bispecific antibodies. Such molecules are formed by choosing the two antibodies: one localizing and one effector. These antibodies are deconstructed enzymatically, and two monovalent Fabs of the separate antibodies are recombined by reestablishing the interheavy chain disulphide bonds. Thus we have prepared a novel bispecific molecule with one arm being the monovalent Fab of a glycoprotein IIb/IIIa receptor blocker and the other the monovalent Fab of an antibody (AP1)

against expressed tissue factor. In vitro and in vivo testing of this molecule suggests it retains the properties of both parents and has enhanced effect on retention of the glycoprotein IIb/IIIa receptor blocker compared with the receptor blocker alone.

Methods

Murine antirabbit GPIIb/IIIa receptor antibody (AZ-1) was conjugated with the antirabbit tissue factor antibody (AP-1) using chemical recombination of antibody fragments. The activity of this novel bispecific product was tested using platelet aggregometry and in a clotting assay designed to measure the inhibitory effects on platelet GPIIb/IIIa receptors and tissue factor respectively. In an ex vivo model the conjugate was delivered to rabbit aorta segments using a microporous infusion catheter (Cordis Corporation, US). The conjugate was mounted in a perfusion circuit to measure retention over 12 hours compared with the original anti-GPIIb/IIIa antibody.

Results

The conjugate inhibited adenosine diphosphate (ADP) induced platelet aggregation almost completely at a concentration of 45 μg per 108 platelets. It also prolonged tissue factor induced clotting time fourfold at a concentration of 6.5 μg/mL. The retention of the conjugate was enhanced; 21.8% remained in the vessel segments after 12 hours compared with 10.6% in the control group ($P < .05$).

Conclusion

It is possible to construct a conjugate of anti-GPIIb/IIIa and tissue factor antibodies that retain biological function and have together enhanced retention in the vessel wall.

Ongoing in vivo studies and future trials will be presented.

Our goal is to identify for any specific process a new antigen or exposure of previously hidden vessel wall component that will allow us to construct molecules that can be delivered systemically in less than normal systemic doses such that they target the pathology, the so called "magic bullet." Dealing with the problems of thrombolysis resistance, thrombolytic failure, and reocclusion are the current aims of our studies.

DEVELOPMENTS IN CATHETER TECHNOLOGIES

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Percutaneous coronary interventions, despite the use of endovascular stents, still have limitations due to early thrombotic events as well as late neointimal proliferation.[1-4] Thrombus formation has been aggressively addressed using new oral and intravenous systemic antiplatelet therapy and improved flow with stents.[5-7] Despite these newer therapies there are still major problems such as acute myocardial ischemia and saphenous vein graft degeneration.[8-10] Progressive disease in saphenous vein grafts is often with a friable and thrombotic base that continues to represent a challenging scenario for percutaneous interventions. Local delivery of pharmacologic agents is now possible using catheters specifically designed for regional or site specific delivery.[11,12] These catheters have been developed to allow an increased volume of agent to be infused at a chosen site in an arterial segment. With the use of the properties of passive diffusion or active infusion, these catheters will deliver pharmaceuticals or genes into the arterial wall.

Clinical approaches have used the ability of the new catheters to approach this problem in one of three techniques: (1) low-pressure diffusion, (2) low volume infusion, or (3) direct injection into the artery wall or pericardium.

While each catheter system has its own unique characteristics, the current delivery systems have equivalent efficiencies of approximately 0.1% to 1.0%.[13] While low, this still represents nearly a 100-fold increase over that seen with systemic administration or infusion through a guide catheter. The limiting problem has not been identifying a delivery catheter but determining what pharmaceutical agent should be administered and what specific clinical problem we are treating.

Coronary dissection and thrombus formation resulting in acute and subacute vessel closure remain significant problems.[14] The decision of which agents to use for local delivery centered on those agents with prior FDA approval. The widespread availability of thrombolytic and antiplatelet therapies resulted in the early use of these agents for local administration. The Dispatch catheter was one of the first systems evaluated in the clinical arena as a local administration device for the indication of coronary thrombus. A nondilating catheter that allows antegrade blood flow while the drug infusion was occurring it also offered the opportunity for prolonged administration of agents without causing myocardial ischemia. This catheter was extensively characterized in humans by Camenzind et al[15] in a novel and elegant manner. Using a continuous infusion of tracer that did not have myocardial uptake with rapid clearance (MAG-3), these authors were able to demonstrate that the actual site of administration