that the drug was having a beneficial effect on chronic pain. Meanwhile, no effects of the drug was seen in studies on normal rats, demonstrating that the drug had no effect on acute pain.

'The complete surprise was that we could separate the effects of acute and chronic pain. This is very exciting because people taking the drug for chronic pain will still be able to experience normal stimuli from the environment,' says Brann, suggesting that different muscarinic receptors are involved in different types of pain.

Potential for the future

ACADIA think that their drug candidates will be helpful for chronic pain associated with diabetic neuropathy, herpes lesions, metastatic cancer and pain associated with autoimmune diseases like Guillain–Barré syndrome. They could also have a role in arthritic and lower-back pain.

Research is now aimed at optimizing the candidate compounds to achieve greater potency, better *in vivo* stability and a longer duration of action. ACADIA hopes to start toxicology and safety studies in animals within a year and the company is aware that it is likely to be 18 months or more before they can start human trials

'The ability to develop small drugs that are highly selective for M_1 receptors is itself a significant achievement,' commented Alan Levey, Professor of Neurology at Emory University School of Medicine (Atlanta, GA, USA). 'This has been a goal for many pharmaceutical companies, given the potential use of an M_1 receptor-specific drug for Alzheimer's disease, schizophrenia, pain and other common problems.' He continued, 'ACADIA used a different approach to find small molecules that activate the receptor subtypes. The finding that drugs can be discovered using this strategy sets an important precedent that could lead to the more rapid discovery of many drug candidates that bind selectively to a variety of receptors, and that could be applied to many common medical conditions.'

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Thrombolysis without bleeding

Kathryn Senior, Freelance writer

Plasmin, the activated form of plasminogen (a protein found in normal human plasma), has been previously overlooked as a therapeutic for conditions caused by blood clots. However, a recent preclinical study strongly suggests that plasmin is an excellent thrombolytic agent, with a striking safety profile – better than licensed agents currently in use in the clinic[1].

Lead author, Victor Marder (University of California, Los Angeles, CA, USA), together with colleagues from the Bayer Corporation (Berkeley, CA, USA and Raleigh, NC, USA), used rabbit models of local thrombolysis and fibrinolytic haemorrhage to compare plasmin with tissue plasminogen activator (TPA). TPA is the current standard 'clot-busting' treatment for the management of patients with either an acute myocardial infarction, catheter or shunt, or peripheral arterial occlusion. Although effective, TPA can cause bleeding at remote sites because it spreads systemically, even after local infusion. 'All current thrombolytics are plasminogen activators, of which TPA is the prototype. All have the same problem with bleeding, especially intracranial haemorrhage, which can be fatal,' comments Marder.

Giving plasmin a second chance

During early investigations, plasmin was recognized as a direct fibrinolytic enzyme with a potential clinical application. Desire Collen (University of Leuven, Leuven, Belgium), who is actively involved in the development of recombinant plasmin and derivatives as therapeutic agents, points out in an editorial[2] that, 'Intravenous plasmin for thrombolytic therapy was investigated in several pilot studies in humans in the 1950s and 1960s (Ref. 3).' These showed that plasmin was well tolerated, but the studies were terminated, probably because of a lack of understanding of the kinetics of plasmin inhibition by anti-plasmin, and the unavailability at that time of local catheter delivery. Subsequently, the plasminogen activators streptokinase and urokinase were discovered; both can induce plasmin generation locally within

 1359-6446/01/$ – see front matter PII: S1359-6446(01)02109-2

a thrombus and plasmin was side-stepped in favour of these highly effective enzymes. 'However, in light of the problems with plasminogen activators, we decided to look again at plasmin. The presence of an 'anti-plasmin' in the blood could actually be a useful mechanism to provide safety from haemorrhage, rather than a problem to surmount,' says Marder. 'The working hypothesis is that purified plasmin, totally devoid of plasminogen activator, binds to and dissolves clots when delivered locally. When excess plasmin escapes into the circulation, it is rapidly deactivated by anti-plasmin, thereby avoiding risk of bleeding at vascular injury sites elsewhere in the body,' he explains (Fig. 1).

Supporting data

This study assessed purified plasmin for thrombolytic efficiency and for the risk of bleeding using two standard animal models. In a rabbit model of thrombosis, plasmin and TPA were applied as an intra-arterial infusion into an experimentally thrombosed abdominal aorta. Under conditions of unimpeded blood flow, an equivalent level of clot dissolution and flow restoration was achieved in the thrombosed vessel with 4 mg kg^{-1} of plasmin and 2 mg kg^{-1} of TPA. When the blood flow through the aorta of the rabbit was restricted to reduce local plasminogen supply but to allow plasmin and TPA infusion to still take place, plasmin gave better results than TPA. Another rabbit model, the ear-puncture rebleed model, was used to assess the safety of plasmin versus TPA. In this model, a single 3.5 mm full-thickness puncture is made at sites in the rabbit's ear 30 or 10 min before either plasmin or TPA is infused into the external jugular vein. When similar doses of the two compounds were used (between 2 and 4 mg kg^{-1}), TPA induced rebleeding in a dose-dependent manner from previous puncture sites in nine of ten animals. By contrast, none of the animals given plasmin experienced rebleeding[1].

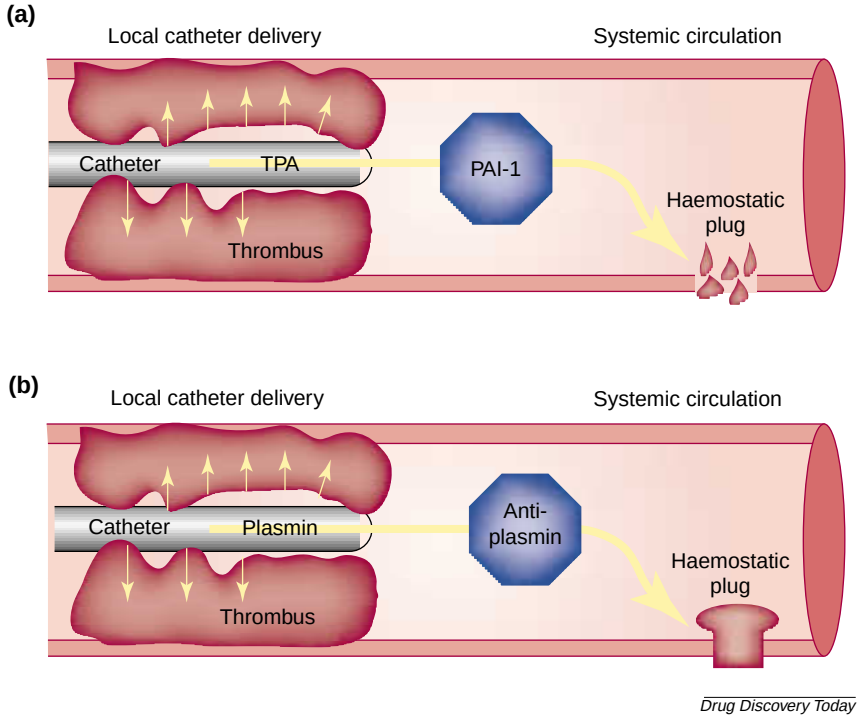

Figure 1. Schematic representation of local and systemic effects of **(a)** tissue plasminogen activator (TPA) and **(b)** plasmin. Local delivery of both TPA and plasmin can induce thrombolysis with local delivery. TPA activates fibrin-bound plasminogen to plasmin. Plasmin has an enzymatic effect after binding to fibrin. However, in the systemic circulation the results are different. TPA exceeds the neutralizing capacity of plasminogen activator inhibitor-1 (PAI-1) and binds to fibrin in haemostatic plugs. Plasmin formation results in fibrinolytic bleeding. By contrast, plasmin is neutralized by anti-plasmin and does not bind to fibrin in haemostatic plugs, therefore, maintaining haemostasis. Figure reproduced, with permission, from Ref. 1.

Collen agrees that 'the data seem convincing and the extrapolations reasonable.' Bayer is also encouraged by the results obtained in the collaborative study. 'These preclinical animal studies demonstrate that plasmin represents a safe and effective thrombolytic agent that does not induce complications such as haemorrhage or antibody response,' says Steve Petteway, Head of Research and Technology at Bayer Biological Products (Research Triangle Park, NC, USA). However, Collen also points out that the plasmin production and purification method employed in the study seems too complicated for large-scale use and warns that it will be necessary to find an effective expression system to enable this large and complex molecule to be produced in large amounts by recombinant DNA technology. 'Intact plasminogen cannot readily be expressed in an active form in common eukaryotic expression systems, because of the presence of intracellular plasminogen activators,' he says. Efforts are ongoing to produce shorter-chain derivatives that retain fibrinolytic activity but which can be expressed more easily. 'We have recently expressed a recombinant variant of human microplasminogen in *Pichia pastoris* (a yeast used for large-scale recombinant-protein production) and converted it with high yield to fully activated and stabilized microplasmin (a low molecular-weight derivative of plasmin),' he reports[4]. However, Bayer has every confidence in producing enough plasmin to meet all future clinical need.

Future studies

'The follow-up studies we are engaged in at the moment are very exciting, in that the promise of the first study is still supported. We have done a dose-ranging study in the rabbit and are currently focusing on correlations of laboratory observations of blood-clotting parameters as pertains to bleeding phenomena,' he says. The group also plans to study the interaction of aspirin and heparin with plasmin, as a prelude to eventual clinical trials. 'Hopefully, Phase I studies will be initiated by Bayer in 2002 for eventual use in thrombolysis by regional infusions by catheter for peripheral arterial or graft occlusion and catheter occlusions,' concludes Marder.

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