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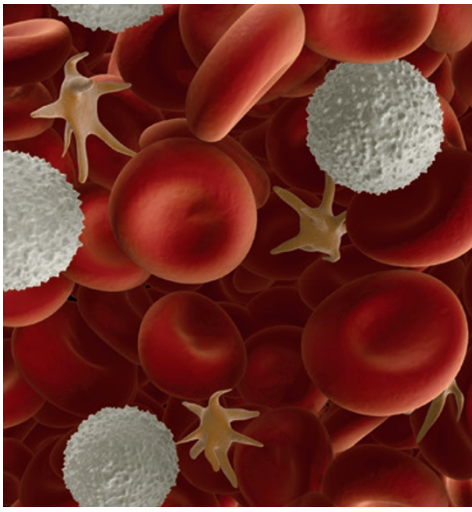
CARDIOVASCULAR DRUGS

Engineered apyrase averts clot formation



Sebastian Kaulitzki/Alamy

The use of existing antiplatelet agents in the treatment and prevention of acute myocardial infarction (AMI) is limited by lack of efficacy against reperfusion injury and increased bleeding risk. Now, Moeckel and colleagues report the development of a novel therapy — an engineered form of the human apyrase ectonucleoside triphosphate diphosphohydrolase 3 (also known as CD39L3) — which effectively and safely prevents thrombus formation, and also decreases infarction size, in mouse and dog AMI models.

P2 purinoceptors are activated by extracellular ATP and ADP, and have a central role in platelet activation and inflammation. Apyrase catalyzes the hydrolysis of ATP and ADP — thereby preventing their interaction with P2 receptors on platelets, endothelial cells and immune cells — to generate cardioprotective adenosine. With this in mind, Moeckel and colleagues set out to optimize the activity of human apyrase.

Previous studies have shown that recombinant CD39 — the first-discovered human apyrase — has antithrombotic properties in experimental animal models, but high doses are required for efficacy and the recombinant protein exhibits aberrant folding. Here, the authors focused on CD39L3, which shares 33% identity with CD39. Following generation of the soluble domain of CD39L3, they constructed variants with the aim of enhancing ADPase activity, while preserving ATPase activity and structural integrity.

This led to the identification of APT102, which exhibited fourfold higher enzymatic activity than the endogenous enzyme and this translated into potent inhibition of ADP-induced aggregation of human platelets, as well as complete reversal of stable platelet aggregation *in vitro*.

Ex vivo analysis of plasma samples taken from dogs that were given an intravenous bolus of APT102 revealed that platelet aggregation was inhibited by more than 90% within 10 minutes of APT102 administration, an effect that was maintained for 24 hours (compared to 60–80% inhibition occurring 4–6 hours following treatment with clopidogrel — a standard antiplatelet agent which acts by specifically inhibiting P2Y12 receptors).

In a model of thrombotic occlusion of a coronary artery in conscious dogs, Moeckel and colleagues next compared the effects of intravenous APT102 versus oral clopidogrel, given in conjunction with standard doses of aspirin and heparin prior to coronary fibrinolysis with recombinant human tissue-type plasminogen activator (rtPA). Reperfusion occurred in all dogs receiving high-dose (1.0 mg per kg) and low-dose (0.25 mg or 0.5 mg per kg) APT102, compared to 75% of those given clopidogrel. High-dose APT102 completely prevented coronary thrombotic reocclusion throughout the 24 hour study duration, whereas reocclusion occurred in all clopidogrel-treated dogs. In addition, high-dose and low-dose APT102 decreased myocardial infarction size by 81% and 62%, respectively, relative to clopidogrel-treated dogs.

Importantly, after clearance of heparin and rtPA from the circulation, bleeding times promptly returned to baseline levels in APT102-treated dogs, whereas bleeding time in clopidogrel-treated dogs continued to increase. Furthermore, no bleeding from surgery sites occurred in dogs treated with APT102.

Finally, in a mouse model of myocardial ischaemia–reperfusion injury caused by transient coronary artery occlusion, APT102 significantly reduced infarct area by 51% and 52% when administered either 5 minutes before 60 minutes of ischaemia or 10 minutes before reperfusion, respectively, without an increase in bleeding time, whereas clopidogrel was not protective in this model and increased bleeding time. Surprisingly, combination of APT102 with clopidogrel significantly attenuated clopidogrel-induced bleeding time prolongation.

These findings support further development of APT102, a novel strategy for AMI adjunctive therapy that not only protects against rethrombosis and reperfusion injury but also attenuates bleeding caused by current antithrombotic drugs.

Sarah Crunkhorn

ORIGINAL RESEARCH PAPER Moeckel, D. *et al.* Optimizing human apyrase to treat arterial thrombosis and limit reperfusion injury without increasing bleeding risk. *Sci. Transl. Med.* **6**, 248ra105 (2014)