MedicalXpress

Scientists find a key protein that allows Plavix to conquer platelets

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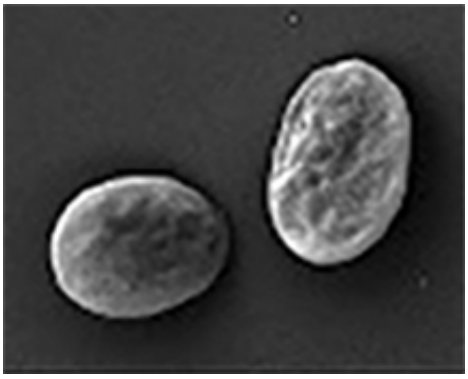


Non-activated platelets are pictured. Credit: Bergmeier lab

Researchers at the UNC School of Medicine have found that the blood platelet protein Rasa3 is critical to the success of the common anti-platelet drug Plavix, which breaks up blood clots during heart attacks and other arterial diseases.

The discovery, published in the *Journal of Clinical Investigation*, details how Rasa3 is part of a cellular pathway crucial for platelet activity during clot formation. Understanding the protein's role could also prove vital in the development of new compounds aimed at altering platelet function.

"We believe these findings could lead to improved strategies for treatment following a heart attack and a better understanding of why

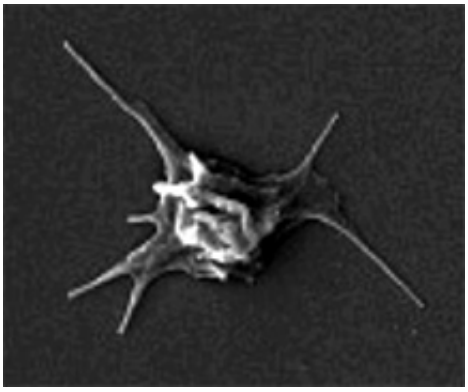
people respond differently to anti-platelet drugs, such as aspirin and Plavix," said Wolfgang Bergmeier, PhD, professor of biochemistry and biophysics, member of the McAllister Heart Institute at UNC, and senior author of the paper.

The research, which was conducted in mice, may also open the door to developing antidotes to Plavix, which was the second-best selling drug in the world prior to its patent expiring in 2012. It is still prescribed under its generic name clopidogrel to millions of people with heart disease, peripheral vascular disease, and cerebrovascular disease. However, the drug's anti-platelet effect increases the risk of bleeding in patients and makes emergency surgery too risky because Plavix affects the ability of platelets to prevent blood loss after vascular injury. An antidote would bypass the need to wait until the kidneys eliminate the drug from circulation.

Since the 1970s, scientists knew that clopidogrel had an anti-clotting effect on platelets. In 2001, they found the compound's target - a cell receptor called P2Y12. As Plavix was developed into a multi-billion-dollar drug, scientists still didn't know how this receptor communicated with other proteins in the cell pathways important for platelet activation. This also meant they didn't know why people responded differently to the drug.

Researchers have since learned that the receptor P2Y12 communicates with a small protein called Rap1, which is like a cellular switch. In platelets, this switch is typically off, which keeps platelets in a non-sticky state. In this quiet state, the 2.5 trillion platelets can patrol blood vessels and arteries without sticking to the endothelium - or inside wall - of, say, a coronary artery. If there's a problem in the endothelium, the Rap1 switch is flipped and platelets morph into super sticky cells that clot fast to keep blood from gushing into tissue.

This is crucial when we have a severe injury or even a cut. But this clotting also happens during a heart attack, when a massive clot is the last thing a person with heart disease needs.



An activated, sticky platelet is shown. Credit: Bergmeier lab

In the arteries that feed blood to the heart, plaque builds over time. But this buildup isn't typically the cause of heart attacks; they occur when the plaque ruptures and platelets rush in to plug the rupture. This clotting blocks the artery, which blocks oxygen from entering the heart. And that causes the heart attack.

To counteract the effects of the clot, Plavix hits its P2Y12 target to flip the Rap1 switch back to the off position so the platelets return to their quiet, non-sticky state. Aspirin also helps keep platelets from sticking.

Until now, no one knew how hitting the P2Y12 receptor triggered the Rap1 protein to switch off. The experiments conducted by the Bergmeier lab show that the Rasa3 protein is a crucial player in this process.

"Platelets live in unique environment and they need to be very sensitive

to changes in that environment," Bergmeier said. "They are ready to jump into action almost without anything happening. You could say they're in a preloaded state. But for that to be possible, they need a breaking system that keeps the platelets in the off state so that they don't do anything until they absolutely have to."

Rasa3 is a key part of that breaking system, and Plavix makes sure that the break stays on.

Think of a platelet like a circuit with Rap1-GDP representing the off state and Rap1-GTP representing the on state. In between, there are proteins called exchange factors (GEFs), which flip on the platelet's Rap1 machinery. The proteins needed to switch off Rap1 are called GAPs. (see illustration)

Using deep sequencing techniques, Bergmeier's team found that Rasa3 was the only highly expressed GAP gene for Rap1 in platelets. He thought that a malfunctioning Rasa3 protein would lead to platelet activation and clearance from circulation.

His team knocked out Rasa3 in mice to show that the offspring had no platelets and could not survive. The researchers then used mice from The Jackson Laboratory to study platelets in mice with a Rasa3 mutation. These mice had 3 to 5 percent of the typical platelet count. Bergmeier's team found that the rest of the platelets were being activated and cleared from circulation.

When the researchers disabled the major GEF proteins, the platelet counts rose to normal amounts in the mice. This showed that a tightly controlled balance between GEF and GAP proteins, especially Rasa3, is vital for platelet activity.

At the sites of vascular injury there's a shift in this balance inside a

platelet that makes the cells very sticky. Plavix ensures that Rasa3 cannot be turned off in platelets; the drug irreversibly limits the cell's ability to stick. It keeps the cell's breaking system perpetually on.

"These experiments show that this Rap1 GEF-GAP pathway is crucial for platelets to jump into action to plug a hole in the endothelium," Bergmeier said. "And now we know that Rasa3 is a critical negative regulator, a break, on the process."

Bergmeier added, "We have good reason to believe that the Rap1 switch, controlled by the same GEF and GAP proteins, also regulates the active state of human platelets. We expect this research will provide critical information for improving anti-platelet therapies, possibly including approaches that eliminate some of the patient-to-patient variability and the increased bleeding risk associated with current anti-platelet drugs."

Provided by University of North Carolina Health Care