Scientist discovers natural molecule indirectly prevents stable clot formation

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A scientist from The Scripps Research Institute has identified a new role for a natural signaling molecule in preventing blood clot formation. The molecule could become a target for the development of novel and cost-effective treatments for blood clotting diseases such as Hemophilia A.

The findings, from a study by Scripps Research Assistant Professor Laurent O. Mosnier, were published in a recent edition of Journal of Biological Chemistry.

The study focused on Platelet Factor 4 – a small cytokine (intracellular signaling molecule) released during platelet aggregation.

Based on Platelet Factor 4 effects on another coagulation protein, it was thought that Platelet Factor 4 could potentially stimulate activation of thrombin-activatable fibrinolysis inhibitor (TAFI) – an enzyme (soluble protein) that protects clot longevity, making clots last longer and preventing excess bleeding; TAFI is like a hardener that is added to the mortar used between the bricks in a brick wall, without which the mortar would never completely solidify, and the wall would never be solid.

The new study, however, found exactly the opposite role for Platelet Factor 4—inhibition of TAFI activation.

For Mosnier, this finding led to a radical idea—sequestering Platelet Factor 4 using such molecules as heparin derivatives could improve clot stability. Heparin - a highly sulfated or negatively charged

glucoseaminoglycan (polysaccharide or sugar derivative) – is a commonly used anticoagulant. Mosnier, however, was able to modify the compound to have the reverse effect and aid in blood clotting in laboratory tests.

"The idea of using heparin to prevent bleeding in kids [who have bleeding tendencies] would be outrageous because that would just greatly accelerate bleeding," said Mosnier, "Our trick, however, was to modulate heparin's anticoagulant properties. This opens up new possibilities."

Converting Heparin from an Anticoagulant into a Non-Anticoagulant

Heparin's anticoagulant activity is derived from a specific pattern of nitrogen- and oxygen-linked sulfation (or simply negative charges) that is recognized by anti-thrombin – the inactivator of coagulation. However, in addition to binding to anti-thrombin heparin also binds to Platelet Factor 4, which is glittered with positive charge, and they attract one another like magnets.

Mosnier found heparin's anticoagulant activity could be prevented, and its Platelet Factor 4 binding selected for, by selectively removing the N-linked sulfations (and further acetylation). This effectively prevented heparin from being recognized by anti-thrombin and allowed it to instead take the Platelet Factor 4 out of the equation. This resulted in prevention of clot breakdown (fibrinolysis), by allowing TAFI to do its job.

To test the effectiveness of the modified heparin derivatives in enhancing clot stability, Mosnier employed a functional assay called a "clot lysis assay." Using a light scattering technique, plasma was used to

generate a clot, which was degraded. Further modulation of the conditions allowed measurement of clot stability via TAFI activation. Mosnier found that, indeed, the modified-version heparin promoted clot stability.

Toward a Cheaper, Cost-Effective Treatment for Hemophilia A

An optimistic Mosnier admits his new discovery is in its infancy, but hopes it may one day provide an alternative treatment for bleeding conditions such as Hemophilia A.

Hemophilia A, which affects 1 in 5,000 males, is an X-linked genetic bleeding disorder whereby there is a reduced amount or activity of factor VIII. This results in the unstable clots, lacking fibrin – a fibrous clot-forming protein. Currently, the treatment for Hemophilia A is prophylactically taking factor VIII as a medicine to improve clotting. Unfortunately, immunity against factor VIII is a significant side effect.

Mosnier hopes that modification of heparin – which is cheaper than factor VIII and already used clinically – could one day stabilize clots in these patients.

"The next step is to see if the modified compound will improve bleeding complications in the Hemophilia mouse," said Mosnier. "We are still a long way from claiming anything clinically."

His optimism is contagious, however, and it is an exciting time for science in the Mosnier lab.

More information: Professor Laurent O. Mosnier of the Scripps Research Institute was sole author of the study, "Platelet Factor 4

Inhibits Thrombomodulin-dependent Activation of Thrombin-activatable Fibrinolysis Inhibitor (TAFI) by Thrombin." For more information, see www.jbc.org/content/286/1/502. … 9a-b999-0eb4d69e474a

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