Towards a new treatment for arterial thrombosis

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Cardiovascular disease (CVD) is the leading cause of death in developed countries, surpassing cancer. Stroke, myocardial infarction or pulmonary embolism are due to thrombosis, i.e. blood clots that form inside blood vessels and thus, block the blood flow. Scientists from the University of Liège (GIGA), in collaboration with an American laboratory, may have discovered a novel anti-thrombotic strategy to prevent or treat these life threatening diseases. They have recently published their work in Circulation, the Journal of the American Heart Association (AHA) (1).

The GIGA scientists have identified a protein called DUSP3 as a potential anti-thrombotic drug target. This protein is produced inside platelets and plays a positive role in platelet activation, causing blood clot formation. Finding pharmacological agents to block this protein activity will probably be a starting point for the discovery of new anti-thrombotic drugs.

Intuitively, thrombosis is an easy phenomenon to understand: a clot that forms in an artery or a vein that prevents blood flow. It is similar to what happens when there is an accident on a motorway: three lanes are obstructed and hundreds of vehicles are stopped. When a blockage occurs somewhere in the circulatory system, the extent of the resulting damage depends on whether the affected "motorway" supplies the entire country or a small country road leading to a dead end.

We undoubtedly have frequent small asymptomatic blockages which occur somewhere in our circulatory system with no detrimental effects on our health. However, these blockages can be problematic, for example,
when they occur in leg veins leading to the so-called "phlebitis". Fifty per cent of cases of phlebitis are asymptomatic. The other fifty per cent of cases may include symptoms of pain, redness or swelling of the affected leg. Pulmonary embolism is a possible complication of phlebitis when the blood clot is released into the blood stream and migrates to the heart and pulmonary circulation. This can result in breathlessness, chest pain and palpitations. Pulmonary embolism can lead to death. The risk factors of phlebitis can be temporary: long immobilization, an orthopedic surgical intervention, a plane journey or a pregnancy but they can also be permanent: ageing, obesity, heart failure, cancer etc. The most frequent arterial thrombosis is the one affecting the coronary arteries leading to myocardial infarction. If the thrombus/clot reaches an artery that supplies the brain, it can cause a cerebrovascular accident (CVA) or stroke, which consists of a sudden interruption in the blood supply to part of the brain. Deprived of oxygen, brain cells die leading to permanent brain damage with serious consequences resulting in a vegetative state, physical or mental handicap etc. For sure, the pathological process leading to arterial thrombosis is a long and insidious phenomenon. It is due to atherosclerosis, i.e. development of cholesterol-rich atheromatous plaques in the arterial wall. Thrombosis occurs at late stages, when a vulnerable plaque suddenly ruptures. Risk factors for atherosclerosis are hypertension, bad cholesterol, diabetes, smoking or alcohol abuse, in addition to aging.

How does the clot form?

There has been a continued improvement in knowledge about the cellular mechanisms leading to blood clot formation "As soon as a blood vessel wall is damaged", explains Dr Cécile Oury (FRS-FNRS- Research Associate, GIGA-Cardiovascular Sciences at the University of Liege), "blood platelets play a crucial role: a serie of reactions activates platelets so that they stick to the injured area, which result in the formation of a clot to 'plug the hole' in the vessel. This physiological process, called hemostasis, is required for the maintenance of the integrity of the circulatory system; otherwise we would bleed to death after the occurrence of the first hemorrhage. In a normal situation, the clot disappears spontaneously. Thrombosis is the abnormal pathological situation where the clot does not dissipate and form a thrombus". Actually, the most widely used drugs to prevent arterial thrombosis act to limit platelet activation and aggregation. The best known and most popular is aspirin. Taken on a daily basis in small doses, often combined with clopidogrel (marketed under the brand name Plavix), aspirin therapy reduces the risk of cardiovascular events by 25%. "This is not enough", says Dr Souad Rahmouni (FRS-FNRS- Research Associate, GIGA-Signal Transduction at the University of Liege), "in addition, this treatment has serious side effects such as stomach ulcers and excessive bleeding" It is little to say that finding novel anti-thrombotic agents with a more favorable safety profile is a pressing medical need.
Finding a new target

It is in this context that the two scientists from GIGA have identified a new target, a protein called DUSP3. This protein, which is produced inside the platelets, is a member of the "phosphatases" family. Proteins from this family play, usually, an inhibitory role in cellular activity. Surprisingly, and according to the GIGA team’s new findings, DUSP3 acts as a positive regulator of platelet activity: it stimulates, rather than inhibits, platelet activity. To investigate further DUSP3 function in platelets, the scientists used a genetically modified mouse (known as knock-out mice or KO), in this case, DUSP3-KO.

Cécile Oury explains, "We have discovered that platelet activity is inhibited in DUSP3-KO mice, lacking DUSP3 protein. These mice are more resistant to experimentally-induced thrombosis when compared to normal mice. Interestingly, these mice do not bleed more than normal mice, indicating that primary hemostasis mechanisms are preserved. Therefore, if we can pharmacologically block the activity of this protein, we will have found a new and probably safe anti-thrombotic strategy"
Part of the research published in *Circulation* was carried out in the US in collaboration with Dr. Lutz Tautz's team, at the Sanford Burnham Medical Research Institute (SBMRI) of La Jolla, California, where Souad Rahmouni performed her post-doctoral training on the family of protein phosphatases. SBMRI has an important molecular screening center (Conrad Prebys Center for Chemical Genomics) with an industrial capability of drug screening where it is possible to find a needle in a haystack. In this case, the objective was to identify a synthetic molecule among hundreds of thousands that would behave as a "blocking agent" for the enzymatic activity of DUSP3 protein. In other words, a molecule that will bind DUSP3 and prevent it from carrying out its function. The screening performed at Sanford-Burnham Institute was conducted by powerful robots and analyzed more than 300,000 molecules!

Thanks to this screening, Dr. Tautz's team isolated about ten inhibitory molecules specific to DUSP3 protein. These molecules were tested in Liège on isolated platelets from human blood (tests carried out *ex vivo*) in order to verify their impact on platelet activation and aggregation. The dialogue between Liège and La Jolla lasted for nearly two years and resulted in the design of a molecule that reproduced the same effect as the genetic suppression of the DUSP3 protein in mice, i.e., an inhibition of platelet activity. Specificity studies against other members of the phosphatases family were performed and showed that the selected molecule was highly specific to DUSP3. Perspectives are the study of toxicity of this new molecule in mice, which is ongoing at the Sanford-Burnham Institute.

If this study proves to be conclusive, the scientists will verify the efficacy of the molecule in vivo in mice. As in the case of the Knock Out mice, it will be necessary to measure the impact on platelet activity as well as resistance to thrombosis in treated as compared to untreated mice.

Clinical research on humans can only begin if results on animals prove to be conclusive. An eventual treatment against blood clots may be at the end of this road but many years down the line.
(1) Dual-specificity Phosphatase 3 Deficiency or Inhibition Limits Platelet Activation and Arterial Thrombosis.