An effect against thrombosis

8/25/14

Known for its antiangiogenic effects, the 16K PRL fragment of prolactin was, until recently, mainly studied to fight against the development of tumours. The team led by Ingrid Struman, a researcher at the University of Liège's GiGA-Cancer centre, discovered "somewhat by chance" that this fragment also has an effect against thrombosis, i.e. blood clots that can obstruct our vessels. Consequently, this has opened up new opportunities in the study and therapeutic use of this molecule. A path that hadn't yet been examined up until now.

Who hasn't had a cut or a small wound that seemingly wouldn't stop bleeding? Luckily, nature has thought of everything and provided a mechanism allowing animals and humans not to bleed to death at the slightest injury. Indeed, if we cut ourselves, bleeding stops rapidly in most cases thanks to the aggregation of blood platelets and coagulation, which takes place at the point where the blood vessel is damaged. Among other things, coagulation consists of the formation of blood clots composed of a protein known as fibrin. The mass thus created by the blood platelets and fibrin is then resorbed, after repair of the vessel wall, in order to prevent the formation of thrombi. During this process known as fibrinolysis, the enzyme plasmin dissolves the blood clots. If there is a fault in this process, the blood clots can lead to the obstruction of the blood vessel, i.e. thrombosis. Depending on the location of the blood clot, the consequences can be more or less severe, even fatal, if it prevents the blood from circulating in a vital organ.

Fibrinolysis, a well regulated process!

Besides fibrin, blood clots formed as a result of a wound contain an inactive protein known as plasminogen. It is transformed into plasmin by activators, in particular the tissue plasminogen activator (t-PA), secreted by the vascular endothelium several days after the injury. Once the clot has dissolved, the fibrinolysis process
is regulated to stop and the t-PA is inhibited by... the plasminogen activator (PAI-1). "Today, t-PA, or rather a recombinant form of this protein, is the only molecule used in hospitals as a thrombolytic drug when it is necessary to destroy a large clot that is obstructing a vein or an artery", explains Ingrid Struman, F.R.S.-FNRS research associate and project manager at ULg's GIGA-Cancer molecular angiogenesis laboratory. Ingrid Struman has been studying the 16K PRL fragment of prolactin and its role in angiogenesis for the past ten years or so. "We have known for the past 15 years that 16K PRL has antiangiogenic properties and is capable of blocking the growth of blood vessels", the researcher points out. "Up until now, we have mainly studied it for its antitumoral properties since angiogenesis is essential for tumour growth", she continues. Indeed, it is thanks to the creation of blood vessels in its close environment that a tumour is ensured of the delivery of resources in oxygen and nutrients required for its development.

**From angiogenesis to the dissolving of blood clots...**

Within this knowledge on 16K PRL, Ingrid Struman and her team initiated a fundamental research project aimed at finding the mediator molecule for the antiangiogenic and antitumoral effects of this prolactin fragment. "We performed a yeast library screening within the framework of this project. This technique allowed pieces of genes to be expressed by yeasts and to observe which proteins bind to 16K PRL", Ingrid Struman explains. This is how the researchers identified the protein PAI-1 as the mediator for the effects of 16K PRL. The scientist and her colleagues, in particular doctors Khalid Bajou and Stéphanie Herkenne, undertook a series of experiments with cellular tools and animal models to see whether they could confirm this role of PAI-1.

"We observed that when we invalidated the expression of PAI-1, 16K PRL lost its antiangiogenic and antitumoral effects, which proves that the action of 16K PRL is a result of the intervention of PAI-1", Ingrid Struman reveals. These results have been published in the journal *Nature Medicine*(1). But, besides the discovery of the mediator of the known effects of 16K PRL, this study has allowed researchers to pinpoint another function of the 16K PRL fragment, which was completely unknown until now! "The identification of PAI-1 as the protein that binds the 16K PRL fragment has opened a completely new door to fibrinolysis", the scientist explains.
A new door opens on the functions of 16K PRL

As mentioned earlier, PAI-1 inhibits t-PA in order to halt the fibrinolysis process and therefore dissolve the blood clots formed during coagulation. Considering the role of PAI-1 in this process and its ability to bind 16K PRL, the researchers from Liège thought it would be a good idea to explore a possible function of this prolactin...
fragment in fibrinolysis. "It's something that was never explored before as we didn't suspect any link between PAI-1 and 16K PRL", the researcher continues. The tests for thrombosis carried out on mice models proved conclusive. "When we treated, with 16K PRL, mice in which a blood clot has been induced in the carotid artery, we observed that the clot disappeared far more quickly", Ingrid Struman explains. "The 16K PRL fragment inhibits the PAI-1 protein and consequently, t-PA more efficiently dissolves the blood clots", the scientist adds. This discovery is very interesting since there was no other known natural PAI-1 inhibitor until now... Now they were on this path, Ingrid Struman's team wanted to know exactly which regions of the 16K PRL prolactin fragment were responsible for inhibiting PAI-1. "We worked with smaller peptides from this fragment and we identified interesting regions. A patent was filed following this work", the researcher says. In the mid or long term, depending on the efficacy and toxicity results of the tests on these peptides, they could be used in the treatment of thrombosis.

**When fundamental research finds therapeutic pathways**

This study is a very good example of fundamental research during which researchers attempt to better understand a mechanism, leading to the discovery of a new function of a protein. "We weren't expecting this at all and our discoveries could ultimately lead to a therapeutic application", Ingrid Struman stresses. Although its arrival on the drug market is still far away, the 16K PRL fragment could have a major benefit with its antiangiogenic and thrombolytic effects. Because, as the researcher points out, "many tumour treatments using antiangiogenic therapy cause thrombosis problems in patients". Using an antiangiogenic that also has a thrombolytic effect could therefore allow us to overcome these side effects. "But at this stage, this is still only a hypothesis...", Ingrid Struman emphasises.

One of the next stages of this research is to see whether the 16K PRL fragment is naturally present to regulate the formation of blood clots. Little is still known of the physiological role of 16K PRL today. A study shows that PRL is synthesised and cleaved into 16K PRL by the retina and that these molecules play a role in the prevention of angiogenesis in the retina. It is the only physiological role of 16K PRL to have been demonstrated up until now. Besides this physiological role, various studies have revealed that 16K PRL is associated with effects observed in certain pathologies. This is particularly the case in peripartum cardiomyopathy where an excess of 16K PRL in the heart plays an important role in the development of this disease. (Read the article "Detecting peripartum cardiomyopathy").
Dual function for 16K prolactin

**Angiogenesis**
- Example: Angiogenesis inhibition by 16K PRL is PAI-1 dependent

**Fibrinolysis**
- Example: 16K prolactin accelerates blood flow restoration in a model of arterial thrombosis, this effect is PAI-1 dependent.

16K prolactin inhibits angiogenesis via PAI-1  
16K prolactin improves fibrinolysis via PAI-1

**LEFT:** example of 16K PRL effect on angiogenesis: PAI-1 is required for 16K prolactin (16K PRL) to impair neovascularization in the retina. Isolectin-B4 staining of blood vessels in retinas from postnatal day (P) 4.5 WT and PAI-1-/- neonates mice upon treatment with vehicle or 16K PRL. Scale bar: 500 μm. **RIGHT:** example of 16K PRL effect on fibrinolysis. 16K PRL inhibits the anti-fibrinolytic activity of PAI-1.

a. Representative blood flow recordings (Doppler) of a blood flow restoration model in injured carotid arteries. Five minutes before carotid injury, vehicle (top) or 16K PRL (1.5 mg/kg) (bottom) was administered. Ten minutes after FeCl3 injury-mediated occlusion, a bolus of unfractionated porcine heparin (200 U/Kg) was administered followed by a continuous heparin infusion (70 U/Kg/h). Ten minutes later, a continuous tPA infusion (100 μg/kg/min) was started. b. Reperfusion time calculated as the time interval between tPA administration and blood flow restoration (return of carotid flow to 50% of baseline). A time value of 90 min was used for the conditions where no restoration of blood flow was observed (no lysis). (N=6; * P=0.0022 vs vehicle in WT by Fisher’s exact test).