Proteins present in tick saliva may help us understand and diagnose cardiovascular diseases

Atherosclerosis is the main cause of death worldwide. It turns out a protein called evasin-3, which is present in tick saliva, may be able to help Dr Ingrid Dijkgraaf based at Maastricht University to understand this cardiovascular disease. By attaching a fluorescent marker to evasin-3, the team was able to see inflammation inside arteries, a sure sign of developing atherosclerosis.

Atherosclerosis is the main cause of cardiovascular disease worldwide, causing more than 15 million deaths every year. This serious condition develops when arteries become clogged with fatty substances colloquially known as plaque. It may start at any age, but it's usually associated with damage to the inner layer of an artery typically caused by high cholesterol. Once the damage occurs, cells and other substances clump together and build up inside the artery.

As the condition progresses, these plaques not only cause the arteries to narrow and restrict the blood flow to vital internal organs, but also increase the risk of clots when a plaque ruptures that can potentially block blood flow to the heart or brain.

Despite many research groups devoting their work to understanding this condition, there is still much we don't understand about atherosclerosis.

Dr Ingrid Dijkgraaf and her team including Dr Stepan Denisov and Dr Hans Ippel based at Maastricht University, are particularly interested in studying how a group of small signalling proteins called chemokines are involved in the process from the very start. These proteins play a vital role during the development and growth of an atherosclerotic plaque by recruiting cells to the damaged and inflamed region. Somehow, these cells are attracted by increasing levels of chemokines and then they become trapped in the plaque.

Given their involvement, it's not unreasonable to suggest chemokines as a potential target for new therapies. With this in mind, the dutch researchers want to find potential compounds to suppress chemokine action and stop inflammation. After all, although there are several pharmacological and surgical interventions to treat patients with atherosclerosis, a true treatment to stop the cause of this chronic illness is still lacking.

However, the team obtained some curious results that changed their direction of research. What if besides

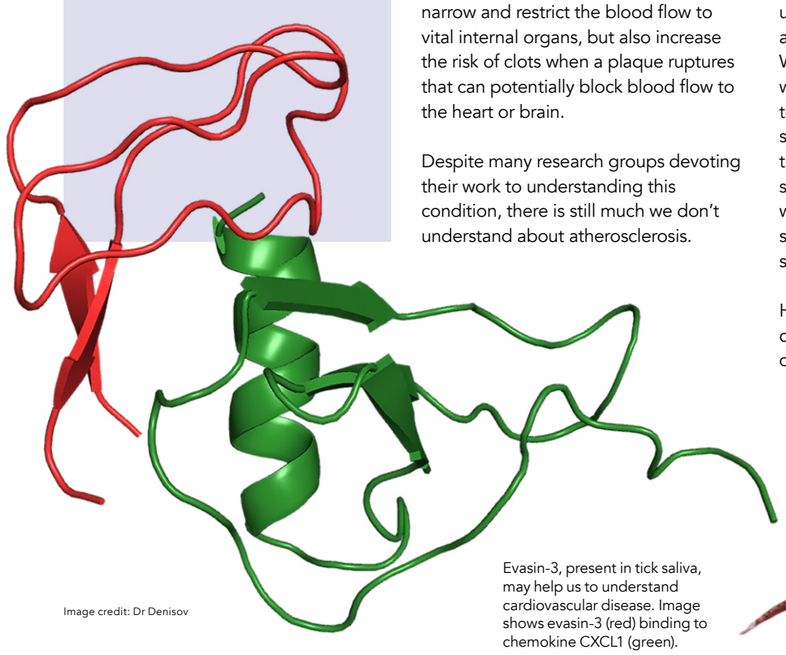

Image credit: Dr Denisov

Evasin-3, present in tick saliva, may help us to understand cardiovascular disease. Image shows evasin-3 (red) binding to chemokine CXCL1 (green).

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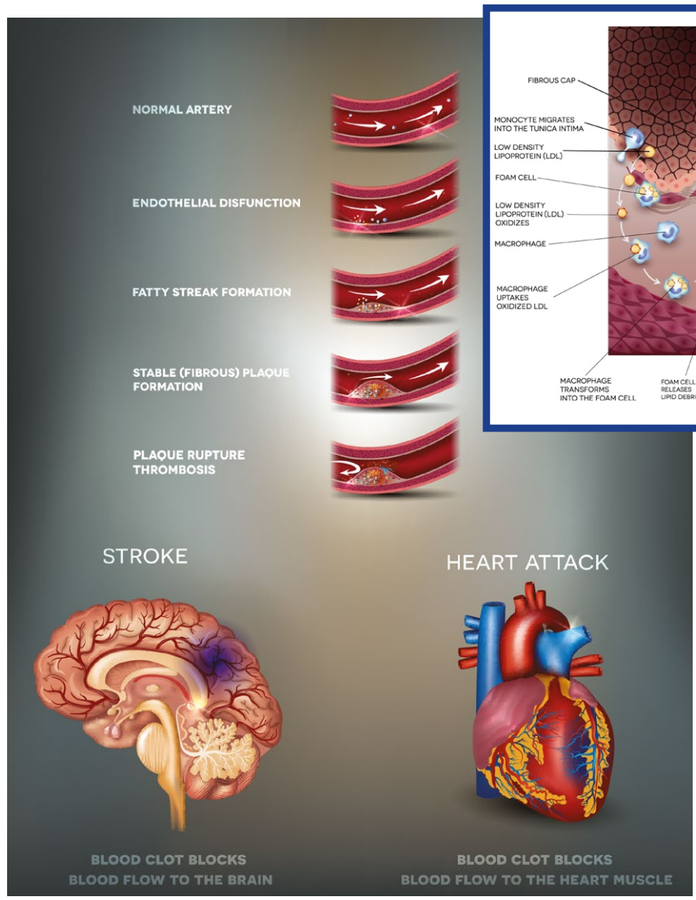

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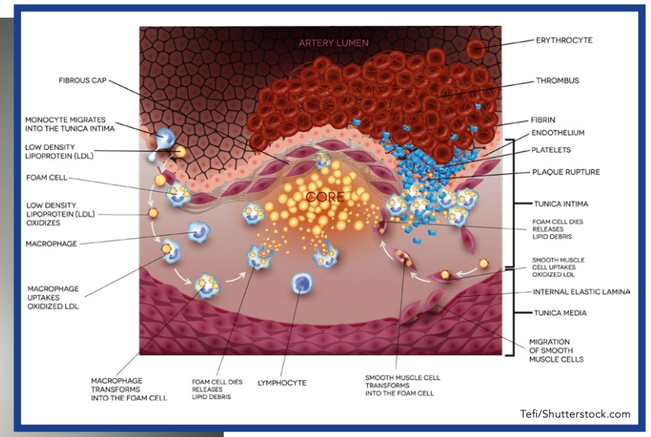

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Chemokines play a vital role during the development and growth of atherosclerotic plaques by recruiting cells to the damaged and inflamed region.

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finding a new treatment, the researchers had found a potential way to diagnose and identify cases of atherosclerosis?

TICKS AND ATHEROSCLEROSIS

For Dr Dijkgraaf, this journey started with an unexpected source: saliva of ticks. These parasites are blood-sucking annoying creatures that can produce numerous proteins in their saliva to avoid being spotted by the immune system of the poor animal that they're biting. These interesting proteins suppress the immune system to make sure that these parasites can feed over a prolonged period of time, before the blood eventually clots.

One interesting family of these tick proteins found in saliva are known as evasins. A protein called evasin-3 is of particular interest to Dr Dijkgraaf, as it can bind chemokines involved in atherosclerosis. This protein is part of the tick's strategy to avoid being spotted. The idea is to neutralise chemokines, which are responsible for recruiting cells of the immune system that protect the host from parasites. "Although ticks are considered nasty parasites, they could help us fight cardiovascular disease, such as atherosclerosis since they have very interesting compounds in their saliva," said Dr Dijkgraaf.

As the first step, the team decided to investigate both the structures of evasin-3 alone and when bound to a chemokine. It turns out evasin-3 contains an important central region that influences the way it binds to other substances. The best way to describe this protein is as dumbbell with a doughnut looped through the middle, where one of the sides of the dumbbell has a rigid structure and the other side is quite flexible. This structure allows evasin-3 to bind to chemokines in such a way that the duo evasin-3/chemokine is still available for possible alternative interactions, while it reduces the attraction of immune cells.

A NEW WAY TO DETECT ATHEROSCLEROSIS

It was exactly this possibility of alternative interactions that triggered a new line of research for Dr Dijkgraaf and her team. They noticed that even when chemokines were bound to evasin-3, they were still able to link to another important player in atherosclerosis: a type of sugars called glycosamino-glycans.

These compounds are part of the body's normal response to inflammation.

Dr Dijkgraaf showed that evasins can spot the presence of chemokines bound to glycosamino-glycans, especially under stressful conditions for the cells which may lead to inflammation.

They're a small component of a healthy artery, but levels increase significantly if the arterial wall is damaged. Essentially, they operate as long hairs to trap lipids and other substances, eventually leading to the formation of plaque characteristic of atherosclerosis.

This discovery opens up an interesting opportunity to use evasin-3 as a way to detect the presence of chemokines bound to these glycosamino-glycans and identify hotspots for the development of inflammation during atherosclerosis. To confirm this possibility, Dr Dijkgraaf used a synthetic version of evasin-3 connected to a fluorescent marker. "By constructing these proteins chemically, we can clarify the molecular mechanisms behind cardiovascular diseases and investigate whether we can use them to reduce or even stop their occurrence," said the researcher.

More insights into cardiovascular diseases will lead to better diagnostics and a possible cure.

Using cells cultured *in vitro*, Dr Dijkgraaf showed that evasins can spot the presence of chemokines bound to glycosamino-glycans, especially under stressful conditions for the cells which may lead to inflammation. The researchers are aware that not all chemokines share the same binding mechanisms, but they believe this is a widespread mechanism and evasin-3 can bind multiple types of chemokines trapped on glycosamino-glycans after stress.

To translate these *in vitro* experiments into a more biologically relevant system, Dr Dijkgraaf also used a whole mouse carotid artery kept in a petri dish.

Similarly to the previous experiment, fluorescent evasin-3 flowing through the artery spotted chemokines attached to glycosamino-glycans. This could be seen as the tissue turned green, clearly identifying spots where inflammation was occurring.

Scientists and physicians already have ways to visualise fatty plaques, but so far there are no methods to spot the actual inflammatory process that makes them extremely dangerous for the patients. To have the ability to "see" where the inflammation is developing would allow doctors to separate patients at a high risk of suffering a cardiovascular disease caused by a blood clot from those with normal artery wall thickening caused by ageing.

WHAT ABOUT HUMANS?

The authors cannot say with 100% certainty that it's the same mechanism in humans, but there's no obvious reason to believe otherwise. After all, mouse and human chemokines are very similar. "At least one mouse chemokine binds evasin-3 and glycosamino-glycans similarly to human chemokines and allows for visualisation of inflammation in arteries," said Dr Dijkgraaf.

Taking into account that synthetic forms of evasin-3 and its variants could be readily produced, this protein represents an attractive candidate for further development of highly accurate and selective imaging procedures to detect early cases of atherosclerosis and other cardiovascular diseases.

In the future, the team hopes to test their method using live animals to find out whether it is still possible to detect inflammation. They also want to replace the fluorescent marker - which can't be seen easily when embedded deep in a tissue - and use a radioactive label instead, which can be used for imaging in humans.

"*In vitro* and *ex vivo* studies demonstrated that evasin-3 might be used as a molecular imaging agent for detection of inflamed endothelium, and thus atherosclerotic plaques noninvasively," said Dr Dijkgraaf. For the researcher, this is essential work as "more insights into cardiovascular diseases will lead to better diagnostics and possibly a cure, so in the future these diseases would not be the leading cause of death in the world."

Behind the Research

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Research Objectives

Ingrid Dijkgraaf explores the proteins in the saliva of ticks as a way to combat cardiovascular disease.

Detail

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Bio

Ingrid Dijkgraaf studied Molecular Sciences with specialisation in Organic Chemistry at Wageningen University. During her PhD at the Radboud University Nijmegen Medical Centre and Utrecht University, she worked on the synthesis and evaluation of radiolabelled peptides for the detection of tumours. She is currently an Associate Professor at Maastricht University.

Funding

- The Netherlands Organisation for Scientific Research (NWO)
- Fulbright Visiting Scholars Program
- Landsteiner Foundation (LSBR)
- MUMC+ Kootstra Fellowship

Collaborators

I would like to thank all my collaborators for their contribution.

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References

- Denisov S, Heinzmann A, Vajen T, Vries M, Megens R, Suylen D, Koenen R, Post M, Ippel J, Hackeng T, and Dijkgraaf I (2020) Tick Saliva Protein Evasin-3 Allows for Visualization of Inflammation in Arteries through Interactions with CXC-Type Chemokines Deposited on Activated Endothelium. *Bioconjugate Chemistry*, 31, 948-955
- Denisov S, Ippel J, Mans B, Dijkgraaf I and Hackeng T (2019) SecScan: a general approach for mapping disulfide bonds in synthetic and recombinant peptides and proteins. *Chem Commun*, 55, 1374-1377
- Denisov S, Ippel J, Heinzmann A, Koenen R, Ortega-Gomez A, Soehnlein O, Hackeng T, and Dijkgraaf I (2019) Tick saliva protein Evasin-3 modulates chemotaxis by disrupting CXCL8 interactions with glycosaminoglycans and CXCR2. *J Biol Chem*, 294, 12370-12379

Personal Response

Do you think this could develop into a standard diagnostic tool to detect atherosclerosis in patients?

"This tick protein could serve as a starting point for the development of an imaging agent to detect this disease. For imaging in patients, radionuclide molecular imaging has some advantages over fluorescence imaging as used in this study. Therefore, we aim to develop an evasin-3-based nuclear imaging compound. However, this means that we need to modify our compound which could affect the biological properties of it and needs further research. In addition, it is important to detect the dangerous, unstable plaques that can rupture and we aim to investigate whether our compound is capable of this."