

Beating diabetes

As understanding of the mechanics behind Type 1 diabetes becomes increasingly blurred, **Professor Christopher Parish** and **Dr Charmaine Simeonovic** have combined the power of two research groups to generate strategies that look towards comprehending and preventing the condition

Could you explain where your research fits in with existing knowledge surrounding Type 1 diabetes?

Type 1 diabetes (T1D) is an autoimmune disease in which the patient's own immune system destroys the insulin-producing beta cells in the pancreas. We have discovered that the insulin-producing cells contain unusually high levels of the complex sugar heparan sulphate (HS) that is essential for their survival. During T1D, the immune system produces the HS-degrading enzyme heparanase that destroys HS within the insulin-producing cells and causes their death. Based on these findings we have shown that heparanase inhibitors (eg. PI-88/Muparfostat), and possibly HS-replacing drugs, can prevent destruction of the insulin-producing cells in mice and thus represent a potential new treatment strategy for T1D in humans.

What was the inspiration and purpose of the research project that led to your discovery?

HS is usually outside cells and acts as a tissue scaffold as well as providing a barrier to unwanted cell entry into tissues. The enzyme heparanase is used by migrating cells to degrade HS and enter tissues, cells of the immune system being particularly effective at using the enzyme. Thus, we predicted that heparanase should play an important role in allowing autoreactive immune cells to enter the pancreas and reach the insulin-producing beta cells.

Did you use any methods or strategies during your research that are particularly interesting or unique?

The non-obese diabetic mouse strain, which spontaneously develops T1D, was essential for us to demonstrate that HS is lost from insulin-producing beta cells during diabetes development and heparanase inhibitors can prevent the disease. We have also developed an *in vitro* culture system for isolated beta cells that has allowed us to show that the addition of HS-like compounds can prevent beta cell death and make the cells remarkably

resistant to reactive oxygen species. In fact, we believe that insulin-producing beta cells, due to their extremely high metabolic rate, require high levels of intracellular HS to protect them from reactive oxygen species damage.

How did you discover that cells producing insulin need HS for their survival?

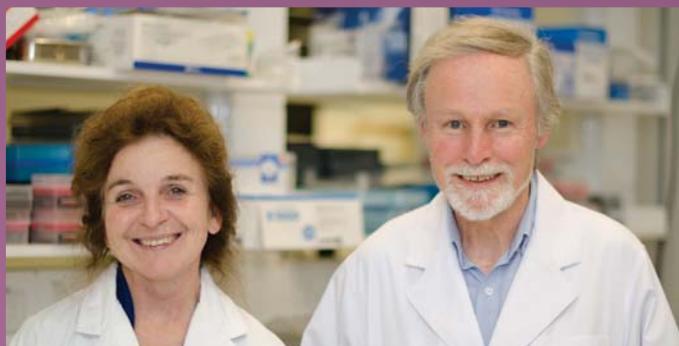
This discovery was highly serendipitous. We were examining sections of pancreas for HS expression and were amazed to find that the insulin-producing beta cells contained extraordinarily high levels of the complex sugar inside them. This was unprecedented as HS is normally concentrated outside cells, not within them. This finding led us to investigate why HS is expressed within beta cells and what happens to the molecule during T1D development.

What effects will treatment with a heparanase inhibitor (PI-88) have on T1D?

Heparanase inhibitors will act at two levels. First, they will inhibit the migration of autoreactive immune cells out of blood vessels, through the pancreas and through an HS-containing barrier surrounding the insulin-producing beta cells. Second, they will prevent immune system-derived heparanase from degrading HS inside the insulin-producing cells and inducing cell death.

All your published work on this topic has involved diabetes in mice. Do your discoveries extend to humans?

We now have a large body of unpublished data showing that human beta cells, like mouse beta cells, contain extraordinarily high levels of HS that is lost from the beta cells in T1D



patients. Also, we have shown that human insulin-producing beta cells are dependent on intracellular HS for their survival, with the HS protecting them from reactive oxygen species damage. Thus, so far, all of our mouse discoveries have been recapitulated in humans.

How do you intend to successfully translate your research and move from bench to bedside with your new medical developments?

We have established a start-up biotechnology company, Beta Therapeutics, that is raising funds to support translation of our discovery into the clinic. Also, the Juvenile Diabetes Research Foundation has been extremely supportive of our research and is keen to aid the clinical development of our new therapies, with potential support from big pharmaceutical companies.

Do you think your work has any relevance to Type 2 diabetes?

Yes we do. We have discovered that HS is lost from insulin-producing beta cells in both mice and humans suffering from Type 2 diabetes. We believe, however, that the HS loss is not heparanase- or immune system-mediated but is due to extreme metabolic stress associated with Type 2 diabetes interfering with the synthesis of HS by the insulin-producing cells. If this view is correct, Type 2 diabetes will need to be treated with HS-replacing drugs rather than with heparanase inhibitors.

Sugar's the remedy

By bringing together in-depth laboratory research and a fiercely collaborative mindset, a team at the **Australian National University** is in a position to shed light on how heparanase inhibitors might be the key to significantly reducing the impact of diabetes

TYPE 1 DIABETES (T1D) is an autoimmune disease caused by the destruction of insulin-producing beta cells in the islets of Langerhans – specialised patches of tissue – within the pancreas by the patient's own immune system. Once these cells have been destroyed, the body can no longer produce insulin, which is vital to control blood sugar levels in the body. There is currently no cure for T1D and sufferers have to depend on daily insulin injections for their survival.

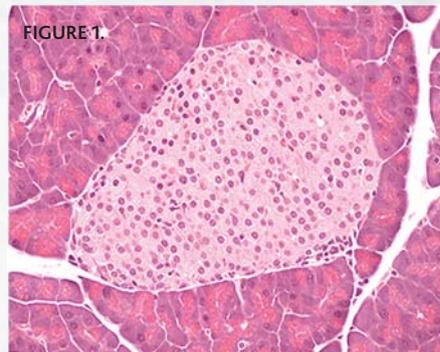
One of the reasons for why a cure has not been found is the uncertainty about how the immune system destroys insulin-producing beta cells. Refreshingly, from the Australian National University (ANU) comes a research group bearing vital new developments.

IDENTIFYING HEPARAN SULPHATE

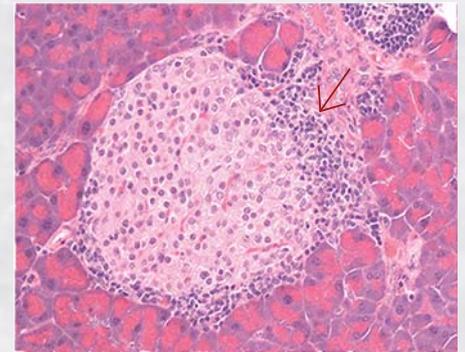
Led by Professor Christopher R Parish and Dr Charmaine Simeonovic based in the Department of Immunology at the John Curtin School of Medical Research, a team of researchers tackled the inadequate knowledge surrounding a condition that affects around 130,000 people in Australia alone. They made the surprising finding that the insulin-producing beta cells contain remarkably high levels of the complex sugar heparan sulphate (HS). They reasoned that the HS may be essential for beta cell survival and, in fact, discovered that beta cell death *in vitro* in both mice and humans correlated with loss of HS from the insulin-producing beta cells.

MINIMISING THE EFFECTS

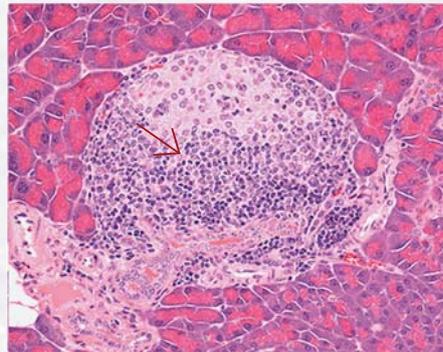
HS is found in all animal tissues and regulates a number of biological processes. However, it is normally present outside cells and not expressed at high levels within cells, as is the case with the insulin-producing beta cells. The researchers resolved this paradox by showing that HS acts as an antioxidant, beta cells being one of the most metabolically-active cells in the body and thus being prone to oxidative damage. The significance of HS must not be underestimated; the team claims that the absence of this particular sugar is one of the most significant reasons for beta cell death. This discovery raised the possibility that beta cells could be kept alive by replenishing HS, as



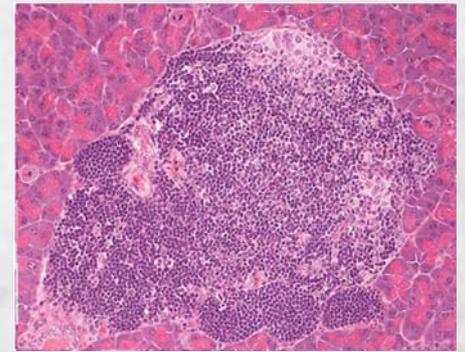
(a) Normal islet of Langerhans.



(b) Early T1D in NOD mice, characterised by accumulation of leukocytes (see arrow) around the islet periphery.



(c) Destructive T1D when leukocytes invade the islet (see arrow) and destroy the insulin-producing beta cells.



(d) Total destruction of islets in T1D.

Simeonovic elucidates: "We have discovered that replacement of HS in the beta cells rescues the cells from dying and prevents them from damage caused by oxidation".

THE THREAT OF HEPARANASE

A crucial question remained to be answered. Do the autoimmune cells that are known to induce T1D deplete HS from the insulin-producing beta cells and if so, how? Key to answering this question was the use of non-obese diabetic (NOD) mice which develop a spontaneous form of T1D that closely resembles that exhibited in humans. The group used this animal model to show that HS levels within beta cells are substantially and progressively reduced during T1D disease onset and progression in these mice. They also showed that during the onset of diabetes, HS within the beta cells is destroyed

by heparanase, an enzyme produced by the autoimmune lymphocytes that are known to induce T1D. Heparanase's part in the development of diabetes is clear: by breaking down HS within the beta cells, it reduces the ability of the cells to resist damaging oxygen species with a resultant loss in insulin production and eventually beta cell death. Once this contributing factor was established in the NOD mice, the study was able to focus on how the effect of the enzyme could be minimised.

PI-88

To prevent the death of beta cells, Parish and Simeonovic strove to develop drugs that both inhibit the heparanase enzyme and replace the intracellular HS that has been destroyed by heparanase. Through *in vitro* studies that tested various inhibitors, they found that the

INTELLIGENCE

ROLE OF HEPARAN SULPHATE AND HEPARANASE INHIBITORS IN THE DEVELOPMENT AND PREVENTION OF TYPE 1 DIABETES

OBJECTIVES

To identify and translate into the clinic a new class of drugs for the treatment and prevention of Type 1 diabetes, namely heparanase inhibitors and heparan sulphate-replacing agents.

KEY COLLABORATORS

Fellow Grant Holders:

Dr Craig Freeman, Australian National University

Professor Gerard Hoyne, University of Notre Dame Australia (Fremantle)

Professor Ray Rodgers, University of Adelaide

Key staff and Postdoctoral Fellows:

Dr Andrew Ziolkowski; Dr Zuopeng Wu; Ms Sarah Popp; Ms Debra Brown; Mr Peter Hamilton; Ms Lora Jensen, Australian National University

FUNDING

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PROFESSOR CHRISTOPHER PARISH is an immunologist and cancer biologist. He currently heads the Cancer and Vascular Biology Group and is Deputy Director of the John Curtin School of Medical Research (JCSMR).

DR CHARMAINE SIMEONOVIC is a Type 1 diabetes researcher with over 30 years research experience in the field. She currently leads the Diabetes/Transplantation Immunobiology Laboratory at the JCSMR.



Heparanase inhibition may be a promising therapeutic strategy for protecting beta cells from heparan sulphate depletion and Type 1 diabetes progression

heparanase inhibitor and HS mimetic PI-88 possesses both of these properties. By testing this therapeutic approach in NOD mice, the ANU group showed that PI-88 indeed had the ability to preserve the islet HS and protect the mice from T1D, results which suggest that heparanase inhibition through drugs like PI-88 may be a promising therapeutic strategy for protecting beta cells from HS depletion and T1D progression.

As well as preventing the progression of T1D in recently diagnosed patients, this approach could also be incorporated into a T1D preventative strategy. While the group is still finalising the best drug for clinical development and has a number of safety trials to undertake, Parish and Simeonovic are optimistic about the potential of heparanase inhibitors in targeting individuals at risk from inherited diabetes. Thus, drugs like PI-88 show signs of not just being able to alleviate the progression of diabetes, but of also having the ability to protect beta cell HS enough to eliminate new cases of T1D.

COLLABORATIVE THINKING

Parish is quick to acknowledge the role of collaborative thinking in his diabetes research with Simeonovic. He is an expert in HS and heparanase research, not diabetes. In contrast, Simeonovic has many years experience in T1D research. It was only when the two researchers combined the expertise of their two laboratories that the role of HS and heparanase in T1D became evident. Unfortunately, such cross-disciplinary research is not common.

Together, the two laboratories have been able to thoroughly interrogate the significance of HS in T1D, and are making steps towards introducing a new drug that will protect beta

cell HS. Speaking of his collaborative work with Simeonovic's lab, Parish observes: "Our success is a classic example of the advantages of cross-disciplinary research".

CLINICAL APPLICATION

The Parish and Simeonovic team is currently mapping out their next steps, which will allow them to take their findings out of the lab and into the clinic. At present, they have identified a desire to target newly-diagnosed diabetics who are experiencing what is described as a 'honeymoon' period of sporadic insulin independence. Judging by the findings so far, this is a key time within the development of the disease, when heparanase inhibitors can be used to rescue the remaining insulin-producing beta cells and potentially halt the effects of autoimmune attack. While this method is not seen as a cure for diabetes, it does offer an appealing solution. By stopping the destructive autoimmune behaviour, the technique will make it possible to resuscitate residual insulin-producing beta cells a number of years after patients first exhibit symptoms of diabetes, lessening a patient's dependence on injections of insulin.

With a proactive approach to taking their findings beyond the lab, Parish and Simeonovic have recently launched Beta Therapeutics, a start-up biotechnology company which is being established in order to make full use of the clinical findings so far and to take advantage of their significant discoveries. Following extensive research surrounding different types of heparanase inhibitors and HS replacers, they are also working out which compounds to take forward to the clinic. Additionally, the duo are looking towards an orally-active-drug which will work to reinforce inhibitors that are currently administered through injection.

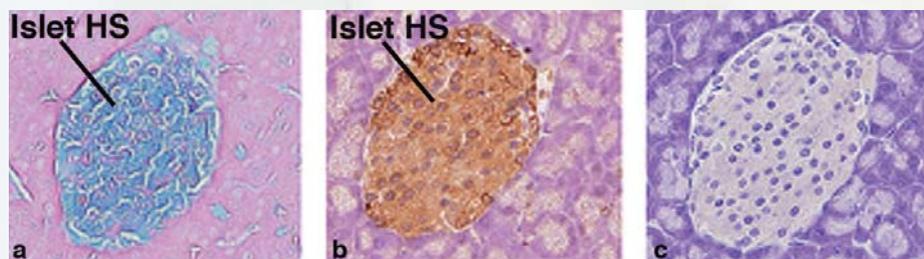


FIGURE 2. Heparan sulphate (HS) is expressed at exceptionally high levels in normal islets in mouse pancreas, as shown by Alcian blue histochemical staining (a) and immunostaining (brown) (b). Normal islet morphology is seen in the absence of staining (c).