COMPOUND COULD HELP DIABETIC PATIENTS WALK TIGHTROPE BETWEEN HYPERGLYCEMIA, HYPOGLYCEMIA

Fran Lowry

In Brief • En bref

A pharmaceutical company is proceeding with clinical testing of a new compound that could make it easier for diabetic patients to maintain good control of blood-sugar levels. The compound, a peptide called amylin, appears to lessen wild fluctuations in blood-glucose levels by slowing the rate of gastric emptying and moderating the amount of postprandial glucose. Fran Lowry interviewed the researcher who discovered the peptide.

Une société pharmaceutique effectue des études cliniques sur un nouveau composé qui pourrait aider les diabétiques à contrôler plus facilement leur glycémie. Le composé, un peptide appelé amyline, semble lisser les fluctuations abruptes de la glycémie en ralentissant le taux de vidange gastrique et en modérant la glycémie postprandiale. Fran Lowry a interviewé le chercheur qui a découvert le peptide.

During an international conference on diabetes in London, England, representatives of a pharmaceutical company announced the discovery of a new compound that could advance the treatment of diabetes mellitus.

The compound, a peptide called amylin, appears to work in a form of partnership with insulin. When diabetic patients eat a meal, their blood-glucose levels soar until the preprandial insulin they have taken kicks in to dispose of the excess. Sometimes, the blood-sugar level goes too low and patients become hypoglycemic. Amylin moderates the amount of postprandial glucose that enters the blood by slowing the rate of gastric emptying. In doing this, it lessens the wild high-low fluctuations of blood-glucose levels that diabetic patients experience.

The company that is producing the peptide, Amylin Pharmaceuticals of Oxford, England, and San Diego, hopes that amylin will help these patients walk the perilous tightrope between hyperglycemia and hypoglycemia, making it easier to control blood-sugar levels. Currently, euglycemia is a goal all diabetic patients are encouraged to seek, in the hope it will stave off the disease's inevitable complications. Unfortunately, keeping the level near normal at all times is difficult, frustrating and often downright impossible.

Amylin may be poised to become a hot commodity, but Dr. Garth Cooper — who actually discovered and painstakingly isolated the peptide, then founded Amylin Pharmaceuticals — may not share in the limelight. No longer part of the company, he wasn't invited to the London conference.

Cooper, professor of biochemistry and nutrition in the Department of Medicine and School of Biological Sciences at New Zealand's University of Auckland, started his career as a biochemist, then became a physician and pathologist. His practice included a number of diabetic patients. After treating them for a long time, he concluded that current knowledge and treatment of the disease left much to be desired. Efforts to achieve good diabetic control underlined just how feeble current treatment modalities were, even in highly motivated patients.

"In theory, tight control is the goal. And the results of the DCCT [Diabetes Control and Complications Trial] published in the *New England Journal of Medicine* [1993; 329: 977–986] did show a reduction in the incidence of diabetic complications in patients who followed the strict regime and kept their blood sugars as close to normal as possible," Cooper told *CMAJ*. "But in practice it's very difficult to live that way all the time — you just can't do it. And that signals the need for something other than insulin alone."

Cooper, who ran a diabetes clinic at Middlesex Hospital in Auckland for a number of years, noticed that once blood-sugar levels were brought down and kept near normal,

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— Dr. Garth Cooper

hypoglycemic reactions would start. "Hypos" are thought to occur because of a lack of epinephrine and glucagon, but Cooper noticed that patients who were normal in these regards still had reactions. He eventually decided that something was missing and decided to find out what it was. "It's very frustrating to treat people with tools you know don't work. I decided to see whether I could make a difference at a scientific level."

Cooper returned to Oxford University, where he had earned a doctorate in science as a Nuffield Fellow, to do fundamental research on the cause of diabetes. His analysis of the literature revealed that amyloid deposits found in pancreata of diabetic patients were held to be irrelevant to the cause of the disease. Cooper chose not to believe this, surmising instead that the amyloid was part of the mechanism that caused diabetes, and that finding out what it was made of ultimately might identify a cause for the disease. "I realized that we had a clue here that hadn't been followed. So I decided to go ahead and isolate and characterize it, and I did, against the advice of some of my seniors."

Those sceptics thought the plan was high risk for two reasons: they doubted whether characterizing the amyloid would give any clues to the cause of the disease and they believed the widely held view that amyloid in the islets of Langerhans was just precipitated insulin. They advised Cooper to forget the whole thing. "They said I could go through 3 years, isolate the amyloid, prove that it was made of insulin, and basically achieve nothing."

Challenging words, surely, for a Nuffield Fellow. The advice was given with the best of intentions, but Cooper stuck to his guns. "My view was that I have one opportunity to make a difference. It may not be insulin, but we have to prove that it is insulin, rather than assume it is. If it really is insulin, it could still be important, but what on earth is [insulin] doing in an amyloid anyway? I was prepared to take the risk."

His gamble paid off. Not only did he prove that the amyloid was not precipitated insulin, he found that it was a completely new hormone. "It was quite a surprise, I must admit," he says.

Isolating it proved very difficult and Cooper admits he almost gave up a number of times. "I used a whole pile of techniques, most of which didn't work. I figured out quite quickly that the stuff was incredibly difficult to deal with."

Cooper worked on pancreata obtained post mortem from patients with adult-onset diabetes. He stained a piece of each pancreas to make sure amyloid was present, then tried to isolate it. The fact that amyloid is present in only a small fraction of the islets of Langerhans, which make up a mere 2% of the entire pancreas, gives some idea of the enormity of the task.

After 9 months, he managed to get amyloid particles that were 50% to 80% pure, but he couldn't dissolve them. "They were extraordinarily resistant to everything I did. All the methods that I tried that worked for other proteins, like detergents, various solvents, heat treatments, you name it, nothing worked. Nothing would dissolve them."

Cooper decided to take the "desperate" step of putting amyloid particles in 70% formic acid, the strongest protein solvent currently known, and then exposing them to high-energy ultrasound. For the first time the particles disappeared. Now he had a solution of the amyloid in formic acid. The next trick was to find it again. "The theory was that this was a protein, but we still didn't know this for sure. We didn't know any of its other properties, we didn't know how big or small it was — we didn't know anything else about it at all."

At the same time, Cooper was putting pancreata from people without diabetes through the same process to compare the extracts. None of the standard electrophoretic or chromatographic methods available for isolating proteins revealed any difference. Cooper eventually developed a procedure using high-performance liquid chromatography, an adapted technique that did reveal a difference.

"I still remember the day it happened. Suddenly there was something there that was different between a diabetic and nondiabetic solution. And once we saw that, we knew we had a method that would isolate the protein that was indicative of the amyloid diabetic extracts — and that protein was amylin." Amylin was then sequenced. Once its chemi-

cal structure was known, it could be reproduced and used in future studies.

The next challenge was to determine what it did. "I discovered amylin in a way that is backwards from the way most hormones are discovered," Cooper explains. "Virtually all hormones that I know of have been discovered as functions — for instance, something that lowers blood glucose — and then scientists go back and look for the structure that does the lowering. This time we came in backwards. We had a structure, but no function."

For Cooper, the clue to amylin's function was that it accumulated in the pancreas of diabetic patients, and presumably had something to do with the cause of diabetes. He eventually found that amylin caused insulin resistance.

It was a very controversial finding — so controversial, in fact, that Oxford diabetologists advised him not to publish it. "They said that people have claimed that they had substances that caused insulin resistance before, but their claims never held up. As a result, they lost their reputations."

He also found that amylin is produced in the same cells that produce insulin. Therefore, when those cells are destroyed in insulin-dependent (type 1) diabetes, both amylin and insulin are lost. This double loss might be a factor in hypoglycemic reactions, Cooper thinks.

When diabetes is well controlled, the peripheral tissues become overly sensitive to insulin, and hypoglycemic reactions become more of a risk — partly because the cushioning effect of high sugar levels is lost. But what if another factor played a role in diabetic reactions?

"Now, suddenly, we have a second hormone that disappears in the type I diabetic, namely amylin, that modulates insulin sensitivity in peripheral tissues. What would happen if we co-replaced amylin with insulin with respect to hypoglycemia?"

Following up on this hypothesis, Cooper conducted a set of experiments in diabetic rats and found that amylin did, in fact, ameliorate the tendency to hypoglycemia. "There are two major problems that are thought to give rise to hypoglycemia," he says. "The first is that skeletal muscle is overly sensitive to insulin. The second is that the liver doesn't produce glucose when it ought to, or as well as it ought to. We found that amylin reversed both of those problems in rats."

Cooper is now studying just how amylin works in the pancreas, skeletal muscle and other tissues. No longer part of the company that he spent 4 years creating, he says he is free to pursue his research without the constraints imposed by working within a corporate environment. Obtaining adequate funding is his only concern now.

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He continues experimental work in his native New Zealand, working with a group of colleagues who share his vision. He also teaches biochemistry to medical students at the University of Auckland, where he has been given free rein to pursue his research and follow his hunches, wherever they may lead. "They've told me just go ahead and do it. Let's see what it is. And that's completely different from the attitude found in a corporate environment."

Above all, Cooper wants to retain the freedom to challenge his own ideas. "It is very frustrating when you find yourself in an environment where you are not allowed to challenge the ideas that you yourself have created."

Cooper believes that the "amylin story" warrants much more investigation before it is touted as a breakthrough for people with diabetes. "I feel that amylin needs much more clinical testing — there is much much more to be done and to be learned about all of this."

More clinical testing is planned. Phase III studies were to begin before the end of 1995 to test the potential of the amylin analogue AC 137 to optimize glucose. The primary endpoints will be lowered hemoglobin A_{1c} and a reduction in hypoglycemic reactions. If these studies are successful, Amylin Pharmaceuticals hopes to file amylin with regulatory authorities in the US sometime in 1997.

A news release from the company says amylin lowered both postmeal glucose concentrations and average 24-hour glucose concentrations in patients with insulin-dependent diabetes who received subcutaneous injections of the peptide.

The company, which clearly thinks amylin shows considerable promise, won't divulge which centres are conducting clinical trials for fear that stock-market analysts will try to contact patients or researchers. "I honestly don't know if we have any Canadian doctors working with amylin," said Richard Krawiec, the company's director of corporate communications, "and I can't tell you where the clinical trials are being done. It's our policy not to reveal where any of the sites are.

"I actually had analysts calling up nurses on a hospital floor, wanting to know how patient so-and-so was doing after receiving amylin. Being a publicly traded company and being a small company, anything like that can be considered a material event [on] which people could trade stocks." ■