Channel-tuning neuropathy

By Michael J. Haas, Senior Writer

An international team has shown that a peptide inhibitor of methylglyoxal treated hypersensitivity to pain in diabetic mice.¹ Although future studies could determine whether the mechanism plays a role in other types of neuropathic pain, the team is now seeking venture dollars to help spin out a new company based on the findings.

Painful diabetic neuropathy (PDN) is a subset of diabetic neuropathy that involves peripheral burning and tingling sensations and hypersensitivity to thermal or mechanical stimuli that are not ordinarily painful.

Hyperglycemia-induced vascular and neuronal changes are known to cause diabetic neuropathy, and past studies have linked neuronal hyperexcitability and increased sodium channel currents to PDN.²⁻⁴ Nevertheless, the precise molecular mechanisms underlying PDN are not well understood.

One possible hyperglycemia-related culprit is methylglyoxal, a toxic by-product of glucose metabolism that has no biological function. In healthy people, it is converted to nontoxic D-lactate by glyoxalase 1 (GLO1), GLO2 and the cofactor glutathione.

In patients with diabetes, serum levels of methylglyoxal can be elevated due to hyperglycemia and deficiencies in GLO1 and glutathione. Previous studies also have shown that, compared with in other cell types, GLO1 is expressed at low levels in the peripheral neurons of normal rats and at even lower levels in those of diabetic rats,⁵ and that the enzyme is necessary for neuronal integrity in roundworms (*Caenorhabditis elegans*).⁶

Collectively, those findings led an international team, which included researchers from the roundworm study, to hypothesize that methylglyoxal might induce changes in peripheral neuronal excitability to cause PDN.

The team first determined that type 2 diabetes patients with foot pain had higher plasma levels of methylglyoxal than diabetic patients without pain and nondiabetic control subjects.

Next, the researchers showed that diabetic mice and methylglyoxal-treated healthy mice exhibited hypersensitivity to thermal and mechanical stimuli, whereas untreated healthy controls did not. In peripheral neurons from the diabetic and methylglyoxal-treated mice, the metabolite had bound the sodium channel Nav1.8 (Pn3; Scn10a) in nociceptive (pain-sensing) neurons to increase excitability (see Figure 1, “PDN: a sodium channel bound to excite”).

The team also found a similar increase in Nav1.8 modifications in the peripheral nerves of patients with diabetes compared with peripheral nerves of nondiabetic subjects.

Additional studies showed that Nav1.8-deficient mice treated with methylglyoxal did not experience thermal and mechanical hypersensitivity, thus confirming that metabolite modification of the sodium channel is responsible for the hypersensitivity.

Lastly, the researchers showed that a methylglyoxal-scavenging peptide decreased thermal and mechanical hypersensitivity in diabetic and methylglyoxal-treated mice compared with an inactive control peptide.

The half-life of the peptide’s therapeutic effect in the mice was about 80 hours, suggesting that once-weekly administration in patients could be sufficient to treat PDN, team leader Peter Nawroth told SciBX. He added that reducing methylglyoxal to—or even below—normal physiological levels should pose no safety problems because methylglyoxal has no biological function.

Nawroth is professor of medicine, acting medical director and chairman of **Heidelberg University Hospital**. His team included researchers from the **University of Erlangen-Nuremberg**, the **University of Bucharest**, the **German Cancer Research Center**, **Riga Stradins University**, the **University of Latvia**, the **Psychiatric Center of South Wuerttemberg**, **The University of Warwick**, the **Albert Einstein College of Medicine of Yeshiva University**, the **Baker IDI Heart & Diabetes Institute**, the **University of Luebeck**, **Heinrich Heine University of Duesseldorf** and **The University of Tennessee College of Medicine**.

Additional team members from **Sanofi’s Sanofi-aventis Deutschland GmbH** subsidiary and the reagent and instrument supplier **Biosistem d.o.o.** jointly performed some of the electrophysiological experiments in mouse neurons and the measurements of mechanical hypersensitivity in mice.

Data were reported in *Nature Medicine*.¹

“These researchers are the first to demonstrate a link between elevated levels of methylglyoxal in diabetes and post-translational modifications of Nav1.8 to increase peripheral nerve excitability and induce hyperalgesia—both regarded as hallmark features of neuropathic pain,” said Maree Smith, professor, reader and head of the pain research group at **The University of Queensland’s School of Pharmacy**. Smith also is director of **TetraQ**, the university’s preclinical drug development CRO.

The study also demonstrates that “treatments that reverse diabetes-induced increases in methylglyoxal formation or that scavenge, degrade or clear the metabolite from the body could potentially treat or prevent painful diabetic neuropathy,” she said.

Ru-Rong Ji, chief of pain research and professor of anesthesiology at **Duke University School of Medicine**, agreed. “Treating neuropathic pain in diabetes patients is a major clinical challenge because current therapies

“Treatments that reverse diabetes-induced increases in methylglyoxal formation or that scavenge, degrade or clear the metabolite from the body could potentially treat or prevent painful diabetic neuropathy.”

**—Maree Smith,
The University of Queensland**

Figure 1. PDN: a sodium channel bound to excite. According to a study in *Nature Medicine*, methylglyoxal (MG) modifies sodium channel NaV1.8 (PN3; SCN10A) in nociceptive neurons to cause thermal and mechanical hypersensitivity in the extremities of patients with diabetes.

Diabetic hyperglycemia raises plasma levels of the metabolite MG [a] that accumulates in nociceptive and other peripheral neurons [b], which express only low levels of glyoxalase 1 (GLO1). The enzyme is required to convert MG to D-lactate.

In nociceptive neurons, MG binds the intracellular portion (inactivation gate) of NaV1.8 [c] and induces unknown structural modifications that inhibit the channel's ability to close in response to increases in the cell's membrane potential.

This increase allows thermal and mechanical stimuli [d] to induce excessive sodium influx [e], resulting in excessive neuronal firing [f] that causes pain [g], even in response to stimuli that are not painful in healthy individuals.

Z123212 (Z212), a small molecule that blocks NaV1.7 (SCN9A) and NaV1.8 sodium channels from **Zalicus Inc.**, is in preclinical development to treat chronic inflammatory and neuropathic pain.

are not very effective and have side effects," he said. "This *Nature Medicine* study offers new targets for treating such pain."

Terry Snutch, CSO of **Zalicus Inc.**, concurred. "It is a nice piece of work with a new and interesting angle on how NaV1.8 could contribute to painful diabetic neuropathy. It also adds to the growing base of evidence that NaV1.8 is involved in neuropathic pain in general."

Later this year Zalicus expects to take its lead compound, Z160, an oral N-type calcium channel blocker, into Phase IIa testing to treat neuropathic pain that is not related to diabetes. The company also has Z123212 (Z212), a small molecule that blocks the NaV1.7 (SCN9A) and NaV1.8 sodium channels, and other undisclosed sodium channel blockers in preclinical development to treat chronic inflammatory and neuropathic pain.

The only two drugs approved to treat pain associated with diabetic neuropathy are Lyrica pregabalin and Cymbalta duloxetine. The former has side effects such as edema, weight gain, concentration and attention deficits and suicidal thoughts. The latter carries a black box warning

for suicidal thoughts and behavior, and has other side effects such as hepatotoxicity and increased blood glucose in patients with diabetes.

Pfizer Inc. and **Eisai Co. Ltd.** market the γ -aminobutyric acid receptor (GABAR) agonist Lyrica to treat neuropathic pain, epilepsy and generalized anxiety disorder (GAD). **Eli Lilly and Co.** and **Shionogi & Co. Ltd.** market the selective serotonin and norepinephrine reuptake inhibitor (SSNRI) Cymbalta to treat diabetic peripheral neuropathic pain (DPNP), other types of chronic pain, anxiety and depression.

Channel surfing

A key question now is whether methylglyoxal's role in pain is limited to diabetic neuropathy.

Ji said the metabolite's effects are probably most important in diabetes-related pain but that "it would also be interesting to know whether methylglyoxal alters NaV1.8 in other nondiabetic pain conditions," such as nerve trauma after surgery or neuropathy associated with viral infection and chemotherapy.

Smith said it is unlikely methylglyoxal-induced modification of NaV1.8 causes hypersensitivity in other types of neuropathic pain. The reason, she said, is that high levels of methylglyoxal develop as a result of hyperglycemia in diabetes.

Nevertheless, "it would be relatively straightforward to examine this question by comparing plasma levels of methylglyoxal in diabetes patients—as measured in this *Nature Medicine* study—with the levels in a range of animal models of neuropathic pain and in patients with neuropathic pain of differing etiologies" such as chemotherapy-induced neuropathy, post-herpetic neuralgia (PHN) and HIV/AIDS-associated neuropathy, she said.

If the team's methylglyoxal-scavenging approach turned out to be relevant only in PDN, "then the clinical hurdles to development of that approach could be high," said Zalicus CEO Mark Corrigan. "Diabetic neuropathy has multiple pathophysiologies, and that variability requires recruiting a large patient population for a clinical study—with associated costs that can be prohibitive for a therapy that has not shown clinical proof of concept in other types of neuropathic pain," he said.

He added that the variability in the pathophysiology underlying PDN has derailed a number of drugs in the clinic. "Consequently, the first indication for a clinical study of a neuropathic pain therapy is usually not diabetic neuropathy," he said.

Ji also wanted to know whether methylglyoxal altered other ion channels involved in diabetic pain, such as transient receptor potential ion channels.

Snutch agreed, adding that modulation of NaV1.8 is "probably not the only mechanism of pain in diabetic patients. For instance, high T-type calcium channel currents are associated with hyperalgesia" in the streptozotocin-induced diabetic mouse model used by Nawroth's team, he said.

Nawroth said the team is now optimizing and testing the methylglyoxal-scavenging peptide in additional animal models of diabetes.

To help develop the peptide as a PDN therapy, the team plans to collaborate with an undisclosed research group that has expertise at making peptides into orally available drugs, he said.

"We are also looking at the effect of methylglyoxal on other channels, not because they have the same importance as NaV1.8 in pain, but more

to understand the crosstalk between channels” and to determine whether modifications to other channels are associated with other symptoms of diabetic neuropathy, he said.

Indeed, this week in *The Journal of Neuroscience*, researchers from the **University of Lausanne**, **University Hospital of Lausanne** and **University Hospital of Bicêtre** reported that low and/or abnormally distributed expression of potassium channel Kv1.2 (KCNA2) in sciatic nerve axons may contribute to PDN in diabetic mice and patients with diabetes.⁷

Nawroth said his team’s findings are patented by Heidelberg University Hospital and are available for licensing or partnering.

However, “we are also looking for venture capital, as we would prefer to start a company” based on the findings rather than out-license the IP, he said.

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