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NEUROBIOLOGY

Life beyond the pain

People with sickle-cell disease experience bursts of debilitating agony from birth. But researchers have made a promising discovery that could ease these excruciating episodes.

BY BIANCA NOGRADY

At first glance, a child with sickle-cell disease may appear healthy. But what you cannot see — and what dominates the child's life — are bursts of searing, crippling pain that strike without warning. These episodes require trips to hospital, and even the highest doses of the most powerful opioid drugs typically provide only temporary, incomplete relief[1].

As children with sickle-cell disease grow older, the pain worsens. They continue to have intense and debilitating acute attacks, and by the time they reach adulthood nearly one-third of them will also experience steady, unrelenting, background pain every day of their lives.

Sickle-cell pain has been thought of as a distress cry of oxygen-starved tissues, as the blood vessels supplying them become blocked and damaged by distorted blood cells. But in the past five years, a proliferation of pre-clinical and early clinical research is forcing a rethink of this assumption. Each new discovery is revealing a jigsaw puzzle in which damaged blood vessels, oxygen-deprived tissues, inflammation, opioid tolerance and hyper-sensitivity are intrinsically connected. Together, these processes present a much more complex picture of sickle-cell pain than researchers had previously believed, one of a damaged and persistently activated nervous system.

A SURPRISE DISCOVERY

In 2013, pain specialist Diana Wilkie, who works at the College of Nursing at the University of Illinois in Chicago, and her colleagues enrolled 18 people with sickle-cell disease in a phase I trial of an agent that they hoped might offer some relief[2]. One of those participants was a woman with sickle cell who had been hospitalised with severe pain 38 times the previous year and could not get further prescriptions of pain-relieving, or analgesic, opioid medication because of limits put in place by her medical insurance. The trial's initial findings were positive: "She had profound analgesia with the study drug", as did nearly half of the study participants, says Wilkie. "She was so happy."

The drug is not a new opioid, or a novel painkiller. It is a widely used antipsychotic medication called trifluoperazine. Why would medicine used to treat the symptoms of schizophrenia have an effect on pain caused by a blood disease? The answer to that question lies in a mystery that Wilkie and her colleagues have been trying to unravel for some time.

The first indication that a non-opioid might mitigate sickle-cell pain came from a study[3] that Wilkie's team published in 2010, in which they asked 145 people with sickle-cell disease to describe their pain using a standard form called the McGill Pain Questionnaire. The researchers expected participants to characterize their pain using terms generally associated with tissue damage caused by oxygen starvation — words such as 'pounding'. But to their surprise, more than 90% also picked descriptors commonly used to describe pain that results from damaged or diseased nerves — words such as 'aching', 'shooting', and 'stabbing'. "We realized that there was a real phenomenon here, and this realization came from the patients' language of their own pain," says Wilkie.

Other evidence was emerging to undermine the conventional belief that sickle-cell pain stemmed from 'vaso-occlusive crises' — the name given to episodes during which blood vessels become blocked by malformed (or 'sickled') red blood cells. Deepika Darbari, a paediatric haematologist at the Children's National Medical Center in Washington DC, says that even people with sickle cell who receive regular blood transfusions to reduce the risk of blood clotting and stroke — and therefore have extremely low levels of sickling — still have pain. "If the

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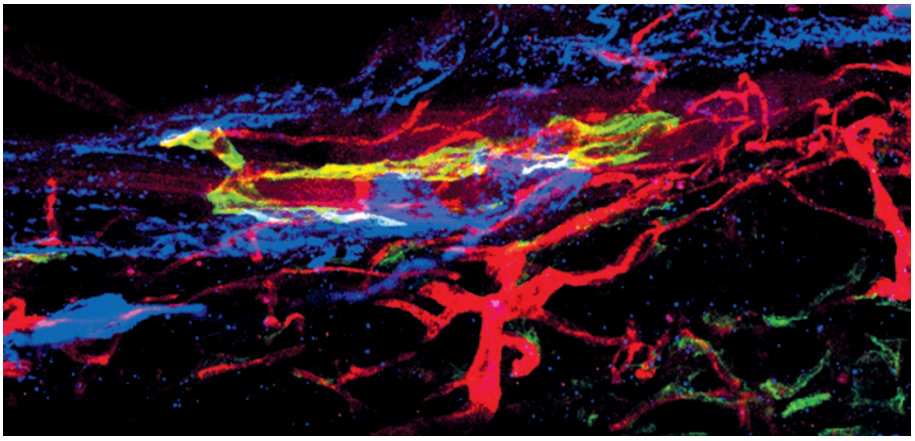

Abnormal clusters of blood vessels (red), nerves (blue) and lymph (green) in the skin of sickle-cell mice.

vaso-occlusive crisis was the only thing causing pain, then these patients should not have any pain because you have taken the sickle cell out of the equation," Darbari says. "So, what else is causing it?"

FEELING SENSITIVE

Mouse studies provide a glimpse into the nervous-system pathology that may underlie some of this long-term pain. Mice cannot communicate pain as directly as a human might, but researchers can still measure an animal's discomfort. They do this by measuring the mouse's grip strength, which is weaker when it is in pain, or by monitoring how quickly it draws its paw away from a stimulus.

To understand the mechanisms of pain in sickle-cell disease, scientists such as Kalpna Gupta, a haematological oncologist at the University of Minnesota in Minneapolis, are examining every level of the pain system in sickle-cell mice and control mice. These studies investigate the entire biological mechanism, from the peripheral nerve fibres in the skin through to the brain and central nervous system, where pain is perceived.

Gupata's work has revealed key differences between the control mice and sickle-cell mice. Not only does the skin of the sickle-cell animals contain fewer nerve fibres, but the nerve fibres that do exist have structures that are arranged in a haphazard and distorted way. The nerves of sickle-cell mice also react much more strongly to two proteins known to cause neural inflammation and increased sensitivity to pain. To Gupta and her colleagues, these observations all point to peripheral nerve damage, possibly the result of inflammation, which in turn leads to sensitization of the nervous system and chronic pain.

Gupta's team has also found[4] increased production of several other substances — the inflammatory cell-signalling molecule interleukin-6, the enzyme COX-2 and a protein of the immune system called toll-like receptor 4 — in the animals' spinal-cord nerves, which they propose would increase nerve inflammation and sensitisation.

Another of Gupta's findings may help to explain why the effects of opioid drugs fade so quickly for people with sickle cell, and why they need higher doses than people with other conditions who have similar levels of pain. Nerves in the skin and spinal cords of the sickle-cell mice showed a reduced level of the μ-opioid receptor, which enables opioid painkillers such as as morphine to take effect. According to Gupta, sickle-cell mice require greater amounts of opioids for pain relief than are normally used in mouse models of pain.

Opioid tolerance in people with sickle-cell disease has been blamed on the fact that the extreme discomfort associated with the condition requires huge doses of opioid medication, leading to an assumption that people become resistant to the effects of the drugs and enter a vicious cycle of escalating doses and continued pain. Yet Gupta's sickle-cell mice had no previous morphine exposure and could not have developed tolerance. She concluded that in mice, opioid tolerance may be another element of sickle-cell biology. Gupta says the findings help to explain the problem of opioid use among people with sickle-cell disease, which is viewed not only as a scientific issue but also as a social one — fear of encouraging addiction may mean that some doctors do not prescribe high doses of opioids to their patients.

> *"There was a phenomenon here, and this came from the patients' language of their pain."*

Further evidence that pain is related to nerve damage came from the discovery that the sickle-cell mice were much more sensitive to cold, heat and touch than control mice. Amanda Brandow, a paediatric haematologist and oncologist at the Medical College of Wisconsin in Milwaukee, has found the same sensitivities to heat and cold — but not touch — in humans with sickle-cell disease[5]. For example, her study participants experienced discomfort at temperatures much closer to the neutral baseline of 32 °C than healthy, race-matched controls.

NEW OPPORTUITIES

One possible explanation for this extraordinary temperature sensitivity is a receptor called TRPV1 (transient receptor potential vanilloid), which is found on certain sensory neurons. When Brandow and her colleagues blocked the TRPV1 receptor in sickle-cell mice, they found that it reversed the animals' sensitivity to touch. The TRPV1 receptor helps to activate an enzyme (CaMKIIα) that is emerging as a major player in sickle-cell pain.

CaMKIIα has been shown to interact with certain nerve receptors called NMDA receptors. This attracted the attention of Jim Wang, a pharmacologist at the University of Illinois at Chicago, and a collaborator of Wilkie's, because NMDAs have a key role in the development of long-term pain. This, combined with the fact that CaMKIIα is expressed in clusters of nerve cells in the spinal cord that process pain signals, suggests that CaMKIIα may be a new target for pain relief in sickle cell and some other conditions.

Instead of creating a new CaMKIIα inhibitor from scratch, Wang wanted to see whether other drugs that are currently available might be suitable. "We started, on a computational model, just looking through the clinically approved drugs that could modulate this enzyme activity," he says.

Wang's systematic search led him to trifluoperazine. His phase I trial of this drug, in partnership with Wilkie and others, halved the pain levels in 8 of the 18 study participants, without any need for extra opioid pain relief. Moreover, Wang says, trifluoperazine may have the added effect of reducing people's opioid tolerance levels so they are once again responsive to those medications. The fact that trifluoperazine worked for some people but not others may have been a result of genetic differences. Or, Wilkie says, it may have been because different patients received different doses, some of which may have been too low to cause a noticeable effect.

The discovery that sickle-cell pain involves neural pathways opens a new chapter in the search for treatments. After decades of opiate-based therapies, Brandow says, the past few years have seen some significant contributions to understanding sickle-cell pain. "The more we understand about the neurobiology, the more people we can get interested in studying it who have experience in pain research," she says. "From that may come novel therapies, which we desperately need." ■

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