THE ENIGMA PILL

Researchers still aren't sure how **ACETAMINOPHEN** works

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OPEN A MEDICINE CABINET in the U.S., and it's likely that acetaminophen, a pain reliever and fever reducer, will be inside. It might be in a pill or a gelcap. It might come in the form of an over-the-counter cold remedy or a prescription medicine such as Vicodin. Acetaminophen is everywhere. So it may come as a surprise to learn that experts aren't quite sure how the drug works.

Researchers have been guessing at acetaminophen's mechanism of action for decades. Some explanations involve chemical messengers of inflammation and pain. Others invoke aspects of neurotransmission in the brain and spinal cord. None of the proposals have accumulated enough supporting evidence to satisfy everyone.

This mystery might seem like minutiae in the grand scheme of things. Acetaminophen has been used clinically for many decades, with more than 27 billion doses sold in 2009 alone. But the drug's well-known danger to the liver makes understanding its mechanism more than a minor detail.

The drug's safety margin—the difference between a safe dose and a toxic dose—is narrow. So it is deceptively easy to take too much and cause a trip to the emergency room, permanent liver damage, or even death. Deaths from accidental acetaminophen poisoning are rare—approximately 150 in the U.S. each year, according to a 2013 investigation by the nonprofit newsroom ProPublica. Even that number is too many to the researchers puzzling over acetaminophen's mechanism of action and seeking safer alternatives.

One mechanism researchers have kicked around is that acetaminophen blocks cyclooxygenase (COX) enzymes. These help to form prostaglandins, which are pain- and inflammation-mediating signaling molecules. This would make acetaminophen similar to aspirin, or to ibuprofen and other nonsteroidal anti-inflammatory drugs (NSAIDs).

But in fact, acetaminophen is its own animal.

In the 1970s, John R. Vane, the Nobel Laureate who cracked the mystery of aspirin's mechanism of action, had proposed that acetaminophen blocked prostaglandin formation in the central nervous system (*Nature* 1972, DOI: 10.1038/240410a0). It was an idea that stuck. In 2002, biochemists reported a new COX variant, dubbed COX-3, that resides mostly in the brain and seemed more sensitive to acetaminophen than were the other COXs (*Proc. Natl. Acad. Sci. USA* 2002, DOI: 10.1073/pnas.162468699). The press, including C&EN, hailed the discovery as the end to the acetaminophen conundrum.

That view soon fell out of favor, however, when researchers realized that the initial experiments, conducted using dog versions of COX, didn't extend to humans. "I think the mechanism is still a mystery," says Brigham Young University's Daniel L. Simmons, who led the COX-3 research. "At least it is to me."

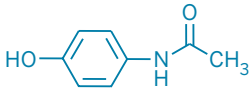
Today the prevailing hypothesis is that acetaminophen needs certain conditions to be a good COX inhibitor—namely, low levels of hydroperoxide-containing compounds (*Clin. Pharmacol. Ther.* 2006, DOI: 10.1016/j.clpt.2005.09.009). Levels of these compounds vary throughout the body, so if this hypothesis is applicable to humans, it would explain a key laboratory observation about acetaminophen: It appears to work better in some types of cells and tissues than others.

As it happens, cyclooxygenase enzymes have two active sites—the peroxidase site, which activates the enzyme from its resting state, and the cyclooxygenase site. Whereas all of the NSAIDs act at the cyclooxygenase site, acetaminophen works on the peroxidase site instead, preventing formation of a key tyrosine radical. The idea is that environments with enough hydroperoxides can spur production of the tyrosine radical even if acetaminophen is around, stymieing the drug's activity.

Vanderbilt University biochemist Olivier Boutaud, whose discoveries contributed to the peroxidase idea, is convinced that inhibiting the COX enzyme alone is enough to reduce fever and relieve pain. "But even though COX is enough, it may not be the whole story," he says.

Peter Zygmunt and his colleague Edward Högestätt at Lund University, in Sweden, are among the experts writing another acetaminophen chapter. They study acetaminophen metabolites and their effects on the central nervous system. One metabolite, AM404, appears to act on COX enzymes as well as on the endocannabinoid system, both of which are involved in pain pathways (*J. Biol. Chem.* 2005, DOI: 10.1074/jbc.M501489200).

Acetaminophen At A Glance



AKA: *N*-(4-hydroxyphenyl) acetamide, paracetamol, APAP

U.S. brand name: Tylenol

First synthesized: 1878

Over-the-counter debut: 1960

Sales: 27 billion doses in 2009

SOURCES: Tylenol.com, ProPublica, Merck Index

& VIDEO ONLINE ACS Reactions takes on the acetaminophen mystery. Share this video with your friends: http://cenm.ag/tylenol

WE ASKED: HOW WELL DO RESEARCHERS KNOW HOW ACETAMINOPHEN WORKS?

"I think the mechanism is pretty clear."

Olivier Boutaud, Vanderbilt

"We have some ideas, but we don't know how it works."

Robert Raffa, Temple

"I think it's becoming clearer."

Garry Graham, University of New South Wales

"I would say poorly."

Daniel Simmons, BYU

"I don't think we've got a complete picture."

Larry Marnett, Vanderbilt

During the past five years, Zygmunt and his collaborators have reported that AM404 activates two different ion channels in the brain and spinal cord—the TRP (transient receptor potential) channels TRPV1 and TRPA1 (*PLoS One* 2010, DOI: 10.1371/journal.pone.0012748). These channels also respond to certain cannabinoids. Zygmunt's colleagues deleted TRPA1 from mice and showed that acetaminophen no longer helped the mice tolerate pain (*Nat. Commun.* 2011, DOI: 10.1038/ncomms1559).

THE TRP RECEPTORS actually elicit pain and itch in response to irritants such as capsaicin, so activating them might seem like a counterintuitive strategy for dulling pain. But if acetaminophen has taught researchers anything, Zygmunt says, it is that pain relief need not come from the expected pathways.

"I think the endocannabinoid link is pretty important, or at least intriguing enough to consider further," says Lawrence J. Marnett of Vanderbilt University. Marnett and his colleagues have delved into the cross talk between endocannabinoid signaling and COX enzymes. They've learned, for example, that inhibiting COX-2 in mice reduces their anxiety-like behaviors through an increase in endocannabinoid signaling (*Nat. Neurosci.* 2013, DOI: 10.1038/nn.3480). What's more, he says, multiple groups have shown that blocking cannabinoid receptor type 1 prevents acetaminophen from relieving pain in mice (*Eur. J. Pharmacol.* 2006, DOI: 10.1016/j.ejphar.2005.12.015).

Yet another possible target is serotonin (5HT) neurotransmission in the central nervous system. Alain Eschalier, a physician-researcher at France's University of Auvergne, showed that when acetaminophen is given to animals in combination with the $5HT_3$ receptor blocker tropisetron, analgesic effects are suppressed. He and his colleagues have also shown that tropisetron reduced acetaminophen's effects on human volunteers (*Clin. Pharmacol. Ther.* 2006, DOI: 10.1016/j.clpt.2005.12.307). Because tropisetron blocks several receptors in addition to $5HT_3$, it's not yet clear which ones might be involved.

Even more potential targets have been proposed but not as actively pursued. In short, acetaminophen's mechanism seems messy enough to discourage even the most optimistic scientists.

But continuing to study the drug is important, stalwarts say. Simmons thinks so simply because it's taken by such a large number of people. Rather than acetaminophen's known liver toxicity, "I'm thinking about subtle yet unknown effects from taking acetaminophen in high doses, long-term," he explains. "Do we really know enough about a drug that's so commonly used to stop inquiring further?"

Zygmunt, meanwhile, contends that acetaminophen's biggest value is as a tool to reveal new principles about pain relief, even if the question of mechanism is never solved.

And Garry G. Graham, a visiting professorial fellow at Australia's University of New South Wales who has studied acetaminophen, says the work has a chance of leading to a safer replacement, albeit a slim one (*Inflammopharmacol.* 2013, DOI: 10.1007/s10787-013-0172-x). Decades ago acetaminophen successfully supplanted phenacetin, the blockbuster painkiller of its day, because phenacetin was toxic to the kidneys, Graham says. "It's possible to change."

Any hope of someday supplanting acetaminophen is moot without a firm understanding of how it works. But acetaminophen continues to defy categorization, says Robert B. Raffa of Temple University School of Pharmacy. He and others have suggested that acetaminophen indirectly uses communication systems similar to those tapped by opioids such as morphine (*J. Pharmacol. Exp. Ther.* **2000,** *295,* 291). He points out that even though acetaminophen interacts with the COX enzymes, it doesn't have much clinical anti-inflammatory activity, the way ibuprofen or naproxen do. "It's not an opioid, and it's not an NSAID," he says. "The question is—what is it?"

Raffa counters his own question simply by saying that research on acetaminophen is complicated. Experts studying acetaminophen each focus on questions that fit within their own small corners of expertise. "We're only getting various hints from each of the groups," he says, which may all hold some truth. "If we could put it all together, I think we would have the whole story." ■