Building a Better Aspirin

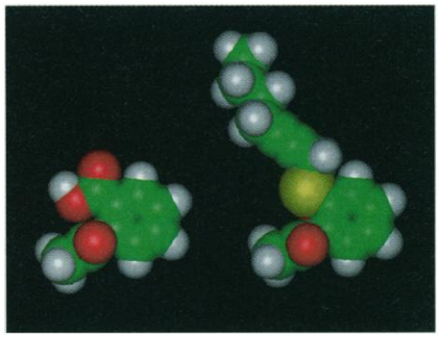
New aspirin-like compounds target a single enzyme to deliver pain relief without stomach and kidney damage. They may also slow the development of cancer and Alzheimer's disease

A century-old wonder drug is about to get even better. Aspirin, first introduced in 1899, relieves pain and soothes aching joints and muscles. But this comfort comes at a cost: Aspirin and all the aspirin-like products, called nonsteroidal anti-inflammatory drugs (NSAIDs), can eat away the stomach lining, causing bleeding or ulcers, and damage the kidneys.

Because these drugs constitute a $14 billion market, pharmaceutical companies have long sought anti-inflammatory compounds without these side effects. The problem with aspirin and its cousins, such as ibuprofen and acetaminophen, is that they aren't sufficiently specific. Their beneficial effects come from their ability to block an enzyme, called cyclooxygenase-2 (COX-2), that promotes inflammation, pain, and fevers. Unfortunately, the drugs are even more effective at inhibiting COX-1, a related enzyme essential for the health of the stomach and kidney. So researchers have been hard at work coming up with compounds that selectively inhibit COX-2, and two may be in drugstores within the next year. But a new wave of COX-2 inhibitors that could eventually be even more potent is also taking shape.

Unlike aspirin, which permanently inactivates the COX enzymes, the drugs in the first wave of inhibitors have only temporary effects. But on page 1268, biochemist Lawrence Marnett and his colleagues at Vanderbilt University School of Medicine in Nashville, Tennessee, report that they have developed compounds that chemically disarm COX-2—and only COX-2—instead of just blocking its activity, as the existing COX-2 inhibitors do. So far, these first irreversible COX-2 inhibitors are no more effective than the compounds now poised to enter the market. But they may be the vanguard of "the next generation" of these wonder drugs, says Stephen Prescott, a molecular biologist at the University of Utah, Salt Lake City. "Eventually all those [NSAID] molecules on the market will become dinosaurs," predicts Philip Portoghese, a medicinal chemist at the University of Minnesota, Minneapolis.

The market for these compounds could be huge, because they are making their debut just as the demand for anti-inflammatory drugs appears ready to burgeon. Over the past decade, epidemiological data have indicated that aspirin and NSAIDs can protect against certain cancers and Alzheimer's disease, and recent laboratory results suggest that COX-2 inhibition is a key factor in these effects (see sidebar). Preventing these diseases would require taking NSAIDs long-term—and selective COX-2 inhibitors seem tailor-made for such use because they appear safer than traditional NSAIDs. Their only apparent drawback is that, unlike aspirin, they can't lower the risk of cardiovascular disease, because that requires inhibiting the clot-promoting effects of the COX-1 enzyme.



Enzyme busters. Altering its chemistry makes the new COX-2 inhibitor *(right)* more selective than aspirin *(left)*.

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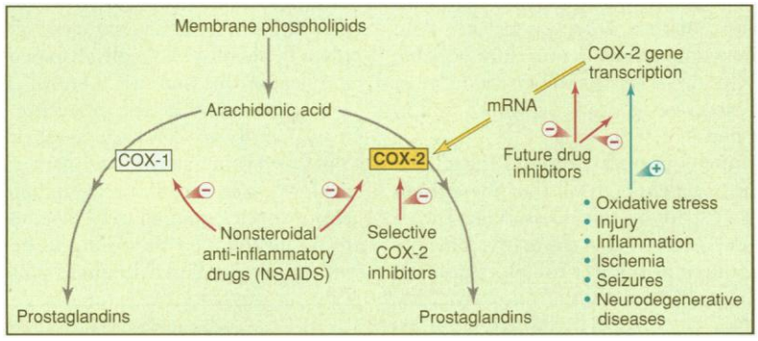

Double action. NSAIDs prevent both COX enzymes from turning arachidonic acid from the cell membrane into prostaglandin hormones; COX-2 inhibitors are choosier.

SOURCE: N. G. BAZAN/LOUISIANA STATE UNIVERSITY MEDICAL CENTER

The search for selective COX-2 inhibitors began in 1991, when researchers first learned of the two COX enzymes. Both enzymes help produce hormones called prostaglandins, although COX-1 is present throughout the body, while COX-2 is made only under certain conditions. But researchers found that only the prostaglandins made by COX-2 lead to inflammation, pain, and fever. COX-1 primarily makes hormones that help keep the stomach lining intact and the kidneys functioning properly.

Efforts to find COX-2 inhibitors heated up in 1996 when several teams determined the crystal structure of COX-2, information that has helped guide drug design (*Science*, 20 September 1996, p. 1660). So far, chemists have found or created about a dozen candidate drugs that block COX-2 alone; another compound that inhibits both enzymes but acts preferentially on COX-2 is already sold in Europe. All those drugs work differently from aspirin in that they bind temporarily to COX-2, thereby blocking its ability to generate prostaglandin. But once the drugs fall off, the enzyme becomes active again. Aspirin, in contrast, transfers an acetyl side group to the COX, permanently disabling it. "It's dead forever," says Prescott. Thus prostaglandin production can resume only after the body produces more of these enzymes.

At Vanderbilt, Marnett and his colleagues set out to make COX-2 blockers that would work in the same way as aspirin. To do this, Amit Kalgutkar in Marnett's group built a molecule that retained aspirin's original acetyl side group but also had a sulfur-containing group—a component of earlier COX-2 inhibitors that helps make those compounds specific for that enzyme. Getting the sulfur group right proved troublesome, however. After several dead ends, Kalgutkar added a sulfur to a ringed molecule and grafted the result to the acetyl group. When he tested this molecule against COX enzymes in a test tube, he found that it preferentially inactivated COX-2, although not as efficiently as he and Marnett would have liked.

Nevertheless, "it proved to us that it could be done," Marnett recalls. The researchers ultimately created a more potent COX-2 inhibitor by adding a tail to the ring, consisting of seven carbons, two of them joined with a triple bond. After building about 70 variations on this chemical theme, they found that the best compounds reduce COX-2 activity by as much as existing, reversible COX-2 inhibitors.

"They've done quite a good job showing it works very well inhibiting COX-2," comments Sir John Robert Vane, a pharmacolo-

Does Aspirin Ward Off Cancer and Alzheimer's?

The new "superaspirins" that are now emerging from the lab (see main text) are likely to have a warm welcome when they reach the market. They appear to offer most of the old benefits of aspirin and its relatives—combating pain, fever, and inflammation—without their stomach-ravaging side effects. And now it seems that both groups of aspirin-like drugs may offer some dramatic new benefits: slowing the progression of cancer and Alzheimer's disease.

The first hints of these benefits came from epidemiological studies, which showed a 45% decrease in deaths from colon cancer and a much reduced incidence of Alzheimer's disease in people regularly taking aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) for arthritis or other conditions (*Science*, 5 July 1996, p. 50). A clue to why NSAIDs seem to inhibit cancer came from biochemist Raymond DuBois and cancer surgeon R. Daniel Beauchamp of Vanderbilt University in Nashville, Tennessee, in 1994. They found high levels of COX-2, an enzyme inhibited by aspirin and its relatives, in about 90% of the colon cancer tumors they examined, a finding that several other studies have confirmed. Monsanto Corp.'s Karen Seibert, a pharmacologist, reports that the COX-2 gene is also overactive in some squamous cell tumors, a skin cancer, and there are slight hints that it plays a role in breast cancer as well.

In 1996, DuBois and his colleagues found a possible role for COX-2 in cancer. When the researchers used genetically engineered cells that made greater than normal amounts of COX-2, the cells became less susceptible to programmed cell death, or apoptosis, which normally removes mutated or damaged cells. "If you look in colon cancer tissue, you find it's blazing with [active] COX-2 gene and protein, and that it's lost programmed cell death," pharmacologist Philip Needleman of Monsanto points out.

Stephen Prescott of the University of Utah, Salt Lake City, thinks COX-2 might also cause trouble because it releases free radicals—such as reactive oxygen ions—as it performs its normal function, generating the hormones called prostaglandins. Because free radicals can cause mutations, they might increase the likelihood of the genetic changes that turn a cell cancerous. And DuBois's team has unpublished data pointing to a third possible mechanism. COX-2, they found, promotes the production of factors that encourage new blood vessels to grow into a tumor, giving it the nourishment it needs to grow.

Why COX-2 inhibitors might protect people against Alzheimer's is less certain. Some researchers think that these drugs simply protect nerve cells against inflammation associated with amyloid plaques, the protein deposits found in the brains of people with Alzheimer's. The drugs might also lower the production of free radicals, suspected of having a role in Alzheimer's brain damage. Others surmise that COX-2 is part of a signaling pathway in nerve cells that—in contrast to the protein's effect on cancer cells—somehow causes apoptosis. In that case, inhibiting the enzyme might spare nerve cells.

Neuroscientist Nicolas Bazan and his colleagues at the Louisiana State University Medical Center in New Orleans have evidence for that scenario. He found that nerve cell injury can turn on the COX-2 gene, increasing the level of COX-2 protein and accelerating apoptosis. When Bazan chemically shut down the COX-2 gene, reducing the amount of COX-2 in cells, "the amount of apoptosis [was] less," he says.

Uncertainties about the exact roles of COX-2 in Alzheimer's and cancer have not stopped researchers from exploring the promise of both traditional NSAIDs and the new COX-2 inhibitors in both conditions. At the American Health Foundation in Valhalla, New York, for example, Bandaru Reddy and his colleagues have shown that rats given celecoxib, a COX-2 inhibitor developed by Monsanto, developed 90% fewer tumors after receiving a chemical carcinogen than control rats did. The tumors that did develop in the treated rats were also less virulent, Reddy and his colleagues reported in the February issue of *Cancer Research*. Reddy's data "are incredibly striking," says Ronald Lubet, a biochemist at the National Cancer Institute (NCI).

Last month, gastroenterologist Russell Jacoby and clinical researcher Carolyn Cole at the University of Wisconsin, Madison, reported other promising results at the annual meeting of the American Association for Cancer Research in New Orleans. Jacoby's group mixed celecoxib with food given to mice bred to develop colon cancer spontaneously. After about 3 months, the mice had only one-third as many tumors as untreated mice, and those that did develop "were almost indistinguishable" from normal gut lining, he reports.

Monsanto is now sponsoring human studies, including one, done in conjunction with the NCI, that is assessing whether celecoxib slows the development of precancerous growths called polyps in people genetically disposed to getting colon cancer. "By next year at this time, we'll know," says Needleman.

The company is also planning to study whether selective COX-2 inhibitors can keep people with Alzheimer's from getting worse, and other studies are planned to see whether the same is true for people who are suspected of having Alzheimer's but who are not yet sick enough to be diagnosed. Again, many researchers are optimistic. "I'd be willing to bet the mortgage that COX-2 [will be involved] in Alzheimer's disease," says Prescott. **–E.P.**

gist and Nobel laureate at the William Harvey Research Institute in London. Marnett, whose university has patented this class of COX-2 inhibitors, sees the compounds as just a "proof of concept." He hopes to team up with a pharmaceutical company to come up with an irreversible drug that binds more avidly to COX-2, making it even more effective at knocking out the enzyme.

Meanwhile, at least two companies, Monsanto Corp. in St. Louis and Merck and Co., based in Whitehouse Station, New Jersey, are well on their way to getting their reversible COX-2 inhibitors to market. Recently, Monsanto finished a study comparing its drug, celecoxib, with existing NSAIDs in 12,000 people with arthritis. "It's fully as efficacious as the NSAIDs" without injuring the gut, says Philip Needleman, a pharmacologist at Monsanto. Merck's product, vioxx, also did well against arthritis and pain in early trials, says Merck's Barry Gertz.

But although the new COX-2 inhibitors seem safe, "the potential long-term adverse consequences are not known," warns John Breitner, an epidemiologist at the Johns Hopkins School of Public Health in Baltimore. He notes that because the drugs seem so safe, people are likely to use them at higher doses for much longer than they would aspirin, with its known risks. The irreversible inhibitors raise an additional concern about how the body might react over the long haul to chemically modified COX-2. Nonetheless, says neuroscientist Nicolas Bazan of the Louisiana State University Medical Center in New Orleans, the search for superaspirins "is a very exciting area of research, one that could benefit many areas of medicine."

–Elizabeth Pennisi