MEDICINE

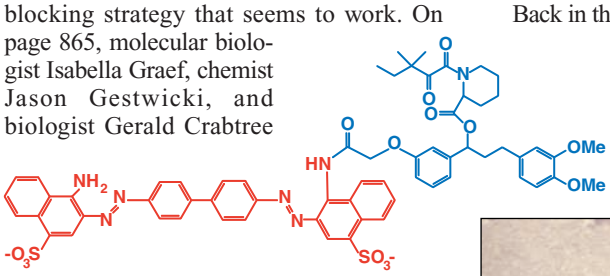
A Wily Recruiter in the Battle Against Toxic β Amyloid Aggregation

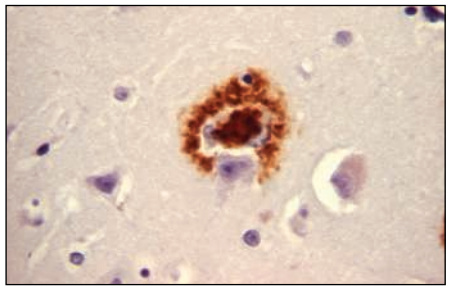
In Alzheimer's disease (AD), large, abnormal clumps of a peptide called β amyloid surround and clog the insides of neurons. These clumps are suspect because they kill cultured neurons, and several human mutations associated with early-onset AD are linked to problematic β amyloid. Hoping to retard the disease, researchers have tried using drugs to block such clumping, but with little success until recently.

The problem: Lilliputian drug molecules are no match for relatively massive amyloid peptides. Using them as blockers is like trying to prevent strips of Velcro from adhering by inserting grains of salt between them. But now Stanford researchers report a new blocking strategy that seems to work. On page 865, molecular biologist Isabella Graef, chemist Jason Gestwicki, and biologist Gerald Crabtree made chemicals that cripple enzymes in bacterial foes by first binding to a giant cellular protein, which then walls off the enzyme from its usual substrate. A prime example is the immunosuppressant FK506. It inhibits the enzyme calcineurin by first recruiting a bulky protein chaperone—a protein that helps other proteins fold—called the FK506 binding protein (FKBP).

Graef was thinking about FK506's mechanism while reading an article about misfolded proteins in spring 2003. She immediately thought: "Why haven't we tried this as a way to block protein aggregation?" She thought β amyloid would be a good test protein because it has been so well studied.

Back in the lab, Graef recruited Gestwicki, who chemically tethered a synthetic ligand for FKBP to Congo red, a dye that sticks to β amyloid but doesn't block clumping except at high concentrations. The resulting small molecule could grab FKBP on one end and β amyloid at the other and thus usher the bulky chaperone in between two amyloid peptides. Gestwicki made several versions of the drug, varying the length and flexibility of the section that linked Congo red to the FKBP ligand.





Bully tactics. A new strategy to prevent clumping of β amyloid (micrograph) combines Congo red (red structure) with another molecule (blue) to maneuver a large cellular protein called FKBP in between amyloid peptides.

describe the synthesis of an ingenious drug that recruits a gargantuan cellular protein to insert itself between two amyloid peptides, preventing the formation of large, toxic β-amyloid clumps.

"It's very clever," says molecular biologist Roger Briesewitz of Ohio State University in Columbus. "By binding a small drug to an endogenous protein, the small drug becomes a large drug that can push away the protein that wants to bind to the drug target."

The method has not yet been tested in animals, and because the current form doesn't cross the blood-brain barrier, it has no clinical use in AD. But if the Stanford team's trick can be parlayed into therapy, it could lead to novel treatments for a variety of disorders—including perhaps other neurodegenerative ailments such as Parkinson's disease—in which protein-protein interactions are thought to play a key role. "We think the idea of fighting protein bulk with protein bulk is going to be general," says Gestwicki. "It's like fighting fire with fire."

The approach has a precedent in nature. For millions of years, soil bacteria have

When added to tubes of β amyloid along with FKBP, Gestwicki's compounds either greatly delayed or completely prevented large clumps of β amyloid from forming, as detected by a fluorescent dye that binds to protein aggregates. The best compound blocked β-amyloid aggregation at concentrations 20-fold lower than any compound previously developed, Gestwicki says, a critical feature for a potential therapeutic. Without FKBP, however, the Stanford drug held no advantage, showing that the chaperone is critical to its modus operandi.

Under an electron microscope, Gestwicki saw that the β-amyloid aggregates that formed in the presence of his drug were much smaller than those in brains with ▶

CREDITS (LEFT TO RIGHT): J. E. GESTWICKI *ET AL.*; UNIVERSITY OF TEXAS MEDICAL BRANCH

ScienceScope

NIH Tweaks Review Criteria to Include Clinical Research

In its first overhaul of grant-review criteria in 7 years, the National Institutes of Health (NIH) has reworded the rules to give more weight to projects that translate research results to patients.

The five grant-review criteria—significance, approach, innovation, investigators, and environment—will now "better accommodate interdisciplinary, translational, and clinical projects," NIH says in a 12 October announcement. For example, "innovation" can include challenging "clinical practice" as well as "existing paradigms." And overall, instead of advancing "a field," the work can "improve clinical decision or outcomes." Reviewers are also asked to review the research teams, not just the lead investigator. The changes, which take effect in January, are part of NIH Director Elias Zerhouni's Roadmap, a set of initiatives aimed at boosting translational research.

Although NIH can't say how the rules might change the mix of basic and clinical research it funds, NIH deputy director for extramural research Norka Ruiz-Bravo is "hopeful" that reviewers "will be even more thoughtful" about these projects.

Clinicians welcome the revisions. "It's going to shift [the mix] some," predicts Herbert Pardes, president of New York–Presbyterian Hospital in New York City, who served on a 1997 NIH panel on clinical research. "The more attention they pay to clinical research, the better."

–JOCELYN KAISER

Russian Parliament Clears Way for Kyoto Protocol

Russia's upper house of parliament was expected to ratify the Kyoto Protocol this week, guaranteeing that the landmark international pact to control greenhouse gas emissions will enter into force early next year. Last week, the Duma, parliament's lower house, voted 334–73 to approve the agreement, and Russian President Vladimir Putin is expected to sign the measure within weeks.

"We'll toast [Russia] with vodka tonight," Greenpeace climate campaigner Steve Sawyer told reporters after the 22 October Duma vote.

After years of debate, Russia's cabinet endorsed the protocol earlier this month (*Science*, 8 October, p. 209). To enter into force, Kyoto needed the backing of nations responsible for at least 55% of 1990 emissions. Russia, with a 17% share, put the pact over the threshold.

–DAVID MALAKOFF

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AD, suggesting that the drug traps the aggregates in an intermediate state. But the researchers still didn't know whether that state was less toxic to cells.

To find out, Graef exposed cultured rat neurons to β amyloid with and without the new drug and FKBP. As expected, β amyloid alone killed the cells. But the drug, along with FKBP, prevented much of the cell death, indicating that the smaller bundles are indeed more benign.

Whether this protection can be extended to animals, let alone humans, remains to be seen. "They've played a creative chemical trick that clearly could be practical," says Peter Lansbury, a chemist at Harvard Medical School in Boston, "but the path from this to an Alzheimer's drug is going to be extremely difficult." One huge problem, Lansbury says, will be finding an alternative to Congo red—which doesn't enter cells or cross into the brain—that targets β amyloid.

The strategy might be easier to employ in other diseases, Lansbury suggests, in which the protein targets are more rigid and stable than β amyloid, which has a floppy, disordered structure. Some oncogenes, for example, work as dimers, so blocking the dimer from forming might lead to a cancer therapy. Viruses and bacteria also enter cells through protein-protein interactions. Says Briesewitz: "If we could use small molecules to disrupt protein-protein interactions, we could target many more biological processes to fight disease."

–INGRID WICKELGREN

NEUROSCIENCE

Prozac Treatment of Newborn Mice Raises Anxiety

The U.S. Food and Drug Administration this month ordered drugmakers to put strong new labels on serotonin-based antidepressants, warning that they may raise the risk of suicidal behavior in children. Now a study by researchers at Columbia University indicates that fluoxetine (the generic name for Prozac), paradoxically, seems to raise anxiety levels in newborn mice.

The study, published on page 879 of this issue, "suggests that fluoxetine and probably other SSRIs [selective serotonin reuptake inhibitors] may have additional unexpected problems," says Miklos Toth, a pharmacology professor at Cornell University's Weill Medical College in New York City. Some scientists caution, however, that the mice in this study were at a much younger developmental age than children likely to be treated for depression.

Fluoxetine is the oldest of the SSRIs and the only one approved for pediatric use. It operates primarily on the serotonin transporter (5-HTT), which is responsible for helping neurons vacuum back up excess serotonin that they have released. By blocking the transporter, the drug enables serotonin to linger in synapses, making more available to be taken up by target receptors.

Chemical imbalance. Mice treated with Prozac as newborns showed reduced exploratory behavior when tested on an elevated maze.

Previous animal research had shown that in early life serotonin acts as a growth factor in the brain, modulating nerve cell growth, differentiation, and migration. Interfering with this function can have behavioral consequences. Mice who have had their serotonin transporters genetically knocked out—and thus reuptake disrupted—exhibit increased depression- and anxiety-related behaviors.

The Columbia researchers, led by psychobiologist Mark S. Ansorge, sought to determine whether fluoxetine would have the same effect as knocking out the two copies of the transporter gene. They bred sets of mice with one, two, or no functioning copies of the *5-HTT* gene. Then they randomly gave either saline injections or fluoxetine—at doses equivalent to therapeutic ones for humans—to newborn mice between 4 and 21 days old in each group. Nine weeks after the last injection, mice were given tests that revealed their emotional states.

As expected, the drug had no effect on the mice lacking any 5-HTT; they already exhibited anxiety. But the two other groups started acting like the 5-HTT–deficient group when they were treated with fluoxetine. In comparison to the saline-treated pups, they showed reduced exploratory behavior in a maze test. They also took longer to start eating when placed in a novel setting and were slower to try to escape a part of the cage that gave them mild foot shocks. All these behaviors are regarded as signs of anxiety and depression in animals.

The authors conclude that disruption of 5-HTT early in brain development affects the development of brain circuits that deal with stress response. Co-author René Hen explains that when serotonin reuptake is blocked, the increased levels in the synapse lead to "abnormal activation [of] a bunch of receptors" during a critical phase of development. "Overstimulation could result in abnormal development" in areas of the limbic system, he says.

The scientists believe that their work could help explain a noteworthy finding announced last year from a longitudinal study of New Zealanders (*Science*, 18 July 2003, p. 386): that people with a polymorphism that reduced their 5-HTT activity were more likely than others to become depressed in response to stressful experiences.

Another implication, of course, is for those exposed to SSRIs at a tender age. The authors say the period of brain development studied in the mice corresponds roughly to the last trimester of pregnancy through age 8 in humans. So, they conclude, "the use of SSRI medications in pregnant mothers and young children may pose unsuspected risks of emotional disorders later in life."

Toth notes that in contrast to humans, a partial deficit (having one defective *5-HTT* allele) is not enough to adversely affect mice's behavior. So "it is possible that humans are more sensitive than rodents to the adverse effect of fluoxetine." But he agrees with Harvard child psychiatrist Timothy Wilens, who says that the "very early exposure calls into question the generalizeability [of these results] to children." Columbia psychiatrist John Mann, who was not associated with this study, adds: "This has nothing to do with the issue of SSRIs in kids because they get the SSRI well after the equivalent period in this study."

Mann says, however, that "this is an important study" because it shows that even transient loss of transporter function during a critical period in brain development may lead to depression in adulthood.

–CONSTANCE HOLDEN

CREDIT: JOHN WOOD

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