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Innovations

Prana Biotechnology, Limited:

Metal Attenuation in the Treatment of Neurodegenerative Disease

In recent months, new energy has centered on the treatment of Alzheimer's disease (AD) and other neurodegenerative diseases; in part, this new interest is sparked by political debates and celebrity appeals for new research measures. "But our work began about 10 years ago when Ashley Bush and I first began studying the interaction of metals with the amyloid-β (Aβ) peptide that makes up the amyloid of the senile plaques seen in Alzheimer's disease," explains Rudolph Tanzi, PhD, professor of neurology at Harvard Medical School and Director of the Genetics and Aging Research Unit at Massachusetts General Hospital (MGH), Boston. Along with Dr. Bush, Dr. Tanzi is also one of two cofounders of Melbourne, Australia-based Prana Biotechnology, Ltd., a company focused on developing new therapies for AD in particular and neurodegenerative diseases in general.

"The idea behind our work was that metals bind the Aβ protein and drive some of the plaque pathology seen in Alzheimer's disease," says Dr. Tanzi. After successful in vitro and animal experiments in the Tanzi and Bush laboratories at MGH, the pair decided to pursue this research in hopes of finding a potential cause for AD. From that effort, Prana was founded. Prana, which has about 15 full-time staff, officially incorporated in Australia in 1997. The seminal science was licensed from MGH and also from the laboratory of Professor Colin Masters at the University of Melbourne, chairman of Prana's Scientific Advisory Board.

MPACs against Neurodegenerative Disease

According to Jonas V. Alsenas, DVM, Prana's CEO, "Overall, our primary purpose at Prana is the development of metal protein-attenuating compounds, or MPACs, for Alzheimer's." Prana's founding science is based on the inappropriate interactions of metals and proteins in the brain. "We believe these interactions cause oxidative stress and oxidative damage and are the primary drivers of toxicity in these neurodegenerative diseases," explains Dr. Alsenas. Imbalances in prooxidant/antioxidant homeostatis cause oxidative stress, leading to toxic, reactive oxygen species, including hydrogen peroxide, nitric oxide, superoxide, and hydroxyl radical. "That science underpins all of the drug development we have done, starting from the work in Drs. Tanzi's and Bush's labs, and also Dr. Masters', and now internally at Prana," says Dr. Alsenas.

He believes MPACs are likely to have a role in treating several disorders, neurodegenerative and degenerative alike. "Many of the diseases we are focused on are associated with aging, but as we become more aware of the usefulness of these compounds and the breadth of toxicity caused by inappropriate metal-protein interactions, it is now becoming evident to us that we are in a position in which we may have assets that may allow us to move past simply age-related diseases to those that are associated much more with oxidative stress," he says. He adds that many age-related disorders, such as AD, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), may develop at least partially from the cumulative effects of oxidative stress.

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Dr. Tanzi explains that a number of metals—notably zinc, copper, and iron—cause the aggregation of this small Aβ protein into amyloid to different degrees. "But it is the so-called reactive metals that can lead to free-radical production, [which is] very dangerous for cells and nerve cells," says Dr. Tanzi. "They include iron and copper. Of those two, copper has a greater attraction to Aβ, so in my opinion, copper is probably the main target."

Though other research groups confirm the detrimental interaction of metals with Aβ, the metal/Aβ hypothesis is far from mainstream. But, recently, scientists seem to agree on two key interactions. First, zinc is required to initiate the aggregation and precipitation of the Aβ into amyloid. And, second, copper acts as a catalyst producing peroxide. "But what these findings imply for therapeutic discovery is where people begin to debate," says Dr. Alsenas.

Attenuators, Not Chelators

"Up until 2000 or 2001, support was lacking for this approach," explains Dr. Ross Murdoch, COO at Prana who oversees drug development and research, including the company's lead MPAC, PBT-1 (clioquinol). But in a 2001 study published in *Neuron*, Bush and colleagues found that clioquinol decreased Aβ peptide sediment by 65% when plaques start to form in 12-month-old mice and by 41% in 21-month-old mice. The authors then concluded that clioquinol inhibits and possibly reverses accumulation of Aβ deposits in the brains of these mice.

The evidence is beginning to suggest that intracellular levels of copper and zinc are depleted in transgenic mice with AD or Alzheimer's-similar pathology. After PBT-1 treatment, the plaques begin to disaggregate and are cleared. "But the metals are not necessarily just cleared out of the body," says Dr. Murdoch. "There has been a misperception that clioquinol and drugs of this class would work as chelators and essentially mop up and clear metals out of the brain. But, instead, we see intracellular levels of the metals rise in the neurons, so that is why we call them attenuating com-

pounds." Clioquinol appears to dissociate copper and zinc from the amyloid to help normalize metal activity and levels in the brain.

"The compounds we are using are not even close; they aren't in the same universe with chelators, which mop up all the metals with an incredible amount of affinity," says Dr. Tanzi enthusiastically. "The advantage of having just enough attraction for copper, zinc, and the Aβ peptide is that PBT-1 and the other MPACs we are developing can get to the right place in the brain and basically pull away the zinc and copper to dissolve it or to prevent it from further aggregating." Chelators have affinity constants in the range of 10^{-20}–10^{-30}. In contrast, PBT-1's affinity constant is about 10^{-11} or 10^{-12}, many dozens of orders of magnitude lower in terms of its attraction than a chelator.

First PBT-1 Human Studies Encouraging

Results from a 36-patient Phase II trial, published in December 2003 in the *Archives of Neurology*, showed that PBT-1 might at least delay AD progression in more severely affected patients. The placebo-controlled, dose-escalation study known as CQAD grouped subjects into two clusters: those with mild-to-moderate disease (less than 25 on the ADAS-cog scale), and those with moderate-to-severe disease (greater than 25 on the ADAS-cog scale). All study participants received baseline Aricept therapy, a commonly used AD drug.

"The analysis showed that in several of the time points assessed in the moderate-to-severe patients, there was a statistically significant 7-point difference compared with the placebo group," says Dr. Alsenas. Looking at biochemical secondary endpoints, PBT-1 appeared to induce statistically significant differences in soluble Aβ plasma levels in the two groups. "This suggested a biochemical basis for what we were seeing cognitively—that PBT-1 was and is disease modifying," according to Dr. Alsenas. "I think we saw a clear signal that in patients who are beginning to decline rapidly, we could show an extremely meaningful difference between them and the placebo group." However, no differences in endpoints were found in the mild-to-moderately affected patients compared with those receiving placebo. "This was not unexpected since patients early in the course of disease are not declining very rapidly," theorizes Dr. Alsenas.

Clioquinol is by no means new, as it was originally marketed globally by Ciba-Geigy from the 1930s–1970s as an antibiotic to treat diarrhea. Clioquinol was removed from the U.S. market in 1971 because of a link with subacute myelo-optic neuropathy seen primarily in Japanese patients. Prana has been pleased with the PBT-1 side effect profile in its trial. "It is all a matter of dose, and though clioquinol was taken off the market decades ago, it was originally used at very high doses," explains Dr. Murdoch. "For Alzheimer's disease, we have reduced that dose."

In October 2004, Prana released results of its CQADEX trial, a 48-week extension study of its CQAD trial. Though only 9 patients completed the full 84-week study beginning with CQAD, it showed that PBT-1 slowed the expected disease progression by about half. In the first half of 2005, Prana plans on initiating a Phase II/III, 435-patient study—PLACQUE (Progression Limiting in Alzheimer's: ClioQUinol's Efficacy)—to examine the effect of PBT-1 in moderate-to-severe Alzheimer's disease patients. Patients will be randomized into placebo and two treatment arms (125 mg and 250 mg BID) and will be treated for 52 weeks.

Dr. Murdoch cautions that it is important to remember that the toxicity associated with AD is in effect the destruction of brain neurons. "So, the concept that a drug can turn toxicity around and assist in the development of new neurons is certainly not what we believe is happening," he cautions. "Instead, we believe these therapies are designed hopefully to stop the disease in its tracks and to break up any amyloid aggregation that has already occurred." But Dr. Tanzi optimistically supports the idea that stopping the disease process puts the brain back on the road to recovery. "If you stop the acute attack on nerve cells— which have an incredible amount of plasticity and compensatory properties—it remains debatable, and controversial, how far you can bring the brain back," he says. He believes that given the ability of a neighboring nerve cell to compensate for a lost synaptic connection, as long as the surviving nerve cells are no longer under attack by toxic amyloid fibrils combining with copper and other reactive metals, "you will be on the road to recovery," he says. "But the extent of that recovery and how optimistic to be is really contentious."

Next up: PBT-2

Although Prana settled a patent dispute over PBT-1 in August 2004 with a Greek company called P.N. Gerolymatos S.A., the company is pursuing next-generation MPACs, including its PBT-2. "Understanding that clioquinol has been used in millions of patients, it still may not be the ideal, so we are developing new compounds that have the same mechanism of action but are structurally different," explains Dr. Murdoch. "PBT-1 is an 8-hydroxyquinoline, and we developed and studied over 300 novel 8-hydroxyquinolone structures before we found PBT-2, which, in our minds, is the optimal candidate." PBT-2 was brought into development just over a year ago, has gone through preclinical toxicity testing, and is expected to be in a Phase I safety trial with healthy volunteers in early 2005. Prana is also developing new compounds aside from 8-hydroxyquiniolone drugs. "Our intention is that those compounds will not only be tested for their efficacy in AD, but they will also be optimized for other diseases, such as Parkinson's," says Dr. Murdoch.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cell.com.**

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