

Regulation of Primate Research

Although the agency has no requirement that nonhuman primates be used for any given trial, let alone studies for longer periods of time, the utility of monkey studies has come to public attention before. The high-profile death of Arizona teenager Jesse Gelsinger during a gene therapy trial (it emerged 3 years ago) followed unreported deaths in monkey models. Now, with the more recent news of serious illness in two patients receiving retroviral gene therapy (see “Gene Therapy on the SCIDs,” page 9), similar questions arise: could human disease or death be prevented by attending more closely, or for a longer period, to the health of primates receiving an experimental treatment?

In an FDA-sponsored conference inspired by the French trial’s adverse events, researchers conceded that most studies of the treatment in large animals had ended after a matter of months, leaving them blind to effects that may develop more slowly. Patient advocates attending the Silver Spring, MD, conference questioned whether such costs are, or should be, prohibitive, when weighed against the possibility of harm to humans. Abbey Meyers, the president of the National Organization for Rare Disorders, raised concerns that patient protection may have taken a backseat to a short-sighted calculation of costs. “What,” she asked the

Patient protection may have taken a backseat to a short-sighted calculation of costs.

panel, “was the cost of saving the money and not following those animals for a longer period?”

Although the NIH has seen large increases in their budget in recent years, Butcher said that it is not translating into similarly large increases in the

amount of primate work done. Cynthia Dunbar, an NIH researcher who has done significant gene therapy work with primates, told the FDA panel that her facility will lose half its space, limiting the ability to take on additional work. “I seriously don’t know if we are going to be able to continue to follow these animals,” she said.

In response to the Gelsinger case, an earlier panel chaired by Daniel Solomon (Scripps Research Institute) came up with a laundry list of reasons to use primates—especially in new vectors that had not been tested in primates before or in cases where other models could not be used. However, they stopped well short of calling for additional levels of testing. “Insisting that every gene therapy trial have a primate model study is something this committee would have to consider very carefully, as the effect on trying to develop trials in academia [could] be devastating,” said Solomon at the November 2000 meeting. Three years later, there remains little talk of implementing such a high standard for preclinical studies.

—Brian Reid

NSAIDS AND ALZHEIMER’S DISEASE: NOVEL ROLES FOR SOME FAMILIAR DRUGS

“The amyloid hypothesis,” says neuroscientist Edward Koo (UC San Diego), “is only a hypothesis. Having said that, there is no other hypothesis that is as good or better.” Indeed, the view that the neurodegeneration seen in people with Alzheimer’s disease (AD) results, more or less, directly from the toxic effects of the β -amyloid peptide dominates the field. As pharmaceutical companies look at predictions of a steadily aging population, drugs that might block amyloid production, and thereby slow the disease process, have attracted enormous interest.

Koo’s statement to the contrary, one other possible explanation for neuronal death in AD, namely brain inflammation, enjoys considerable support. This inflammatory hypothesis has been bolstered by numerous epidemiological studies showing that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the incidence of AD. The most rigorous such study, carried out at the Erasmus Medical Center in Rotterdam, followed nearly 7000 people, who used 16 different NSAIDs for an average of almost 7

years. The therapeutic benefit of these drugs, inhibitors of cyclooxygenases (COX enzymes), seems to speak to a key role for brain inflammation at some stage in the disease process.

This straightforward conclusion may be mistaken, however, since several groups have recently shown that these drugs have previously unsuspected functions, unrelated to COX inhibition, that may fit better with the amyloid hypothesis. Certain NSAIDs, it now appears, can alter amyloid processing in a seemingly benign manner. In addition, naproxen and ibuprofen, among other NSAIDs, are found to bind β -amyloid in vivo and in vitro, preventing its aggregation. Could the drug, therefore, be acting solely through their effects on amyloid? Many researchers remain unconvinced. Gary Landreth (Case Western Reserve University), for instance, cites a variety of other arguments for the inflammatory model of AD unrelated to the epidemiological data. Still, as Landreth himself notes, it remains unclear to what extent the NSAIDs’ protective function is due to their anti-inflammatory actions.

Amyloid Peptides and the Senile Plaque

Amyloid peptides, which make up the bulk of the distinctive “senile plaques” seen in brains of people with AD, are first clipped from a larger transmembrane protein—the amyloid precursor protein (APP). Two enzymes, called β -secretase and γ -secretase, are responsible for the sequential processing of APP to form these shorter peptides. Perhaps because cleavage by γ -secretase occurs within a transmembrane domain (a biological rarity), the peptides that result are not of a single precise length. A 40-amino acid amyloid β -peptide (A β 40) is the most common product formed by sequential action of the β - and γ -secretases. However, a 42-amino acid variant (A β 42), which results when γ -secretase cleaves at a slightly different position, is the peptide found most prominently in senile plaques.

A β 42 is widely suspected to be the toxic peptide that leads to plaque development and neuronal death. Part of the argument rests on genetics. Mutations in two different proteins (presenilins 1 and 2), which are now thought to be part of the γ -secretase complex, can both cause a heritable form of early onset AD. These same disease mutations also increase the amount of A β 42 found in the brain. In addition, A β 42 is much more prone to aggregation (a requirement for plaque formation) than are A β 40 or the other shorter A β peptide fragments. Although it is still controversial whether large deposits of A β (rather than unpolymerized molecules or small aggregates) are harmful to the brain, this difference in the physical properties between the varying length of A β products may be key to A β 42's apparent toxicity.

Targeting γ -Secretase

Regardless of the mechanism by which A β 42 causes trouble, researchers around the world are anxious to find drugs that block its production, and much of this effort has focused on the γ -secretase complex itself. Merck (Whitehouse Station, NJ), Bristol-Myers Squibb (New York, NY), and Eli Lilly (Indianapolis, IN) have all conducted pre-clinical work on γ -secretase inhibitors. However, outright blockade of γ -secretase appears to carry some risk, in part because at least one other protein (the developmentally crucial molecule Notch) is known to be cleaved by γ -secretase. Fears that β -secretase inhibition could be toxic because of anti-Notch activity (or effects on other still undefined γ -secretase substrates) have plagued the field. In addition, Todd Golde (Mayo Clinic, Jacksonville) points out another reason for concern: γ -secretase inhibition might cause accumulation of an APP intermediate form (cleaved by β - but not γ -secretase) with potentially toxic consequences.

A more subtle alternative to inhibiting γ -secretase would be to alter its activity so that it generates less of the A β 42

variant and more of the shorter products. In 2001, Koo's group—and more recently (in a *Journal of Biological Chemistry* paper placed on-line in March 2003) by Takeshi Iwatsubo and colleagues at the University of Tokyo—have found that certain NSAIDs have precisely this effect. Both in vitro and in mice, these drugs (used at relatively high doses compared to typical analgesic uses) can reduce A β 42 levels without affecting A β 40. The drugs also increase the level of a shorter A β species, A β 38, whose toxicity is unknown.

Crucial Differences Among NSAIDs

Only some of the NSAIDs tested showed this activity (see Table 1), despite the fact that all block COX and suppress inflammation. Remarkably enough, the data from the Rotterdam study (which were initially interpreted as broad evidence for the benefits of anti-inflammatory treatment) appear on further examination to support the amyloid hypothesis, Koo indicates. A reanalysis of those data, presented at the 2002 International Alzheimer's Disease meeting in Stockholm, revealed that only those NSAIDs that affect A β 42 formation were significantly associated with Alzheimer's prevention.

Table 1. NSAID Effects on A β 42 Levels

A β 42-Reducing	A β 42-Nonreducing
Ibuprofen	Naproxen
Idomethacin	Aspirin
Sulindac	Celecoxib
	Meloxicam
	SC-560

One major study of NSAIDs in AD is now underway and is expected enroll over 2600 people over age 70. This Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT) was initiated prior to the recent findings on A β 42 levels. It is not designed to test the more recent model, since it will compare the effects of two A β 42-nonreducing drugs (naproxen and celecoxib) to a placebo.

Noting Koo's initial report, the consumer group Public Citizen protested that ADAPT should be halted or altered to instead test one of the NSAIDs shown to have A β 42-reducing activity. Koo, for his part, disagrees, asserting that only trials like ADAPT will clarify whether any particular NSAID can prevent AD. “Our data [suggesting that A β 42 levels might be the key to therapeutic efficacy] are laboratory findings,” he notes, “which may not be physiologically relevant.”

The National Institutes of Health is presently considering initiating a trial to test the preventive effects of ibuprofen, an NSAID implicated by both epidemiological work and the more recent amyloid-related studies.

—Mignon Fogarty