Hormone attack on AD

By Lev Osherovich, Senior Writer

A study by Arizona researchers provides a mechanistic explanation of how gonadal hormones may protect the brain from Alzheimer's disease, thus making a case for developing androgen receptor-activating strategies to treat AD.¹

Although **Ligand Pharmaceuticals Inc.** already has a brain-selective androgen receptor modulator that has completed preclinical development for age-related muscle, bone and sexual indications, the challenge will be to design a trial to catch AD sufficiently early so that treatment still could make a difference.

The findings by a team at **Banner Sun Health Research Institute** build on previous evidence suggesting that altering levels of gonadal hormones can affect AD pathology. Earlier studies in cell culture and in mice showed that natural androgen receptor agonists such as testosterone can lower levels of neurotoxic β -amyloid (A β) in male mice.²

However, in both female and male brains, androgens are converted into estrogens like estradiol by an enzyme called aromatase (see **Figure 1, "Androgens for AD"**). It was thus unclear whether the protective effects of testosterone came before or after it was converted into an estrogen.

"Nobody could tell whether this effect has to do with estrogens or testosterone until now," said team leader Rena Li, senior scientist and director of the laboratory of molecular endocrinology at Banner. The new study reveals that "testosterone is showing benefit on brain action in a gender-specific way," which suggests that strategies for preventing AD may differ between men and women.

Male mice

Li's team began by crossing a widely used AD mouse model with a strain of mice that had a disrupted *aromatase* gene. The resulting animals had only a single copy of *aromatase* and thus showed greater testosterone in the brain than AD mice with two copies of *aromatase*. These heterozygous *aromatase* knockout mice were unable to make detectable amounts of estrogens.

Brains of aromatase-deficient AD mice had fewer signs of pathology than brains of control AD mice with normal aromatase activity. The aromatase-deficient mice also had lower levels of β -site APP-cleaving enzyme 1 (BACE1), a protease that starts the cascade of amyloid- β precursor protein (APP) processing that leads to A β production.

This, in turn, led to lower levels of the neurotoxic A β_{42} fragment of APP that has been linked to AD pathology,³ lower density of amyloid plaques and less pronounced hippocampal degeneration than those in controls.

The team also found that aromatase-deficient mice had higher levels

of neutral endopeptidase (MME; NEP; CD10; neprilysin), an enzyme that degrades A β .

Finally, the aromatase-deficient mice did better than control AD mice in a behavioral assay of learning and memory.

This finding suggests that testosterone may have a two-pronged effect in improving AD: preventing production of toxic APP fragments by BACE1 and stimulating the cleanup of A β fragments by neprilysin.

Results were reported in *The Journal of Neuroscience*.

Although the findings appear to conflict with earlier work by Li's team, which suggested that disrupting aromatase can exacerbate AD pathology in female mice,⁴ Li thinks a closer look reveals concordance between the results.

She said it now appears that both estrogen and testosterone have protective effects. In males, however, the extra testosterone brought about by disrupting aromatase trumps the corresponding decrease in estrogen (see **Figure 1, "Androgens for AD"**).

"In females, when you treat with estrogen, you reduce BACE1 levels," said Li.

Li is now creating an AD mouse with a brain-specific knockout in aromatase to confirm that the effect she observed is exclusively mediated by hormones in the brain. She did not patent her discoveries.

Hormone replacement

Li's findings are in line with previous evidence that pointed to the therapeutic potential of androgen receptor agonists like testosterone, said Christian Pike, associate professor at the Davis School of Gerontology at the **University of Southern California**. In 2006, Pike and colleagues reported that dihydrotestosterone reduced A β levels in male mice with AD.⁵

"By using an aromatase knockout, they're able to prevent the production of estrogens and thus raise testosterone levels," said Pike. "The study confirms that the protective effect of testosterone occurs in the absence of estrogens."

Li's findings suggest divergent approaches for preventing AD in men and women. The former could receive testosterone whereas the latter could be dosed with estrogens. However, safety concerns and questions about when and how to intervene with hormones represent significant hurdles for either approach.

Estrogens once were routinely prescribed as part of hormone replacement therapy for menopausal women. But hormone replacement fell out of favor because of a litany of side effects, including cardiovascular problems and cancer, noted Samuel Gandy, professor of neurology and psychiatry at the **Mount Sinai School of Medicine**. The clinical literature contains ambiguous data about AD progression in women receiving hormone replacement therapy, he said.

Several hormone replacement therapy trials, including the ongoing ELITE (Early versus Late Intervention Trial with Estradiol) study of long-term estrogen therapy in postmenopausal women, have examined neurological endpoints along with their primary focus on cardiovascular endpoints, Gandy noted. The ongoing KEEPS (Kronos Early Estrogen Prevention Study) Cognitive and Affective trial is testing the effect of hormone replacement therapy on memory, attention and executive function in women undergoing menopause.

Figure 1. Androgens for AD. McCallister *et al.* make a case for treating Alzheimer's disease (AD) with androgen receptor agonists.

(I) Ordinarily, androgens such as testosterone are converted by aromatase into estrogens such as estradiol. Lowering aromatase levels using heterozygous deletion of the gene prevents estrogen production and leads to androgen accumulation.

(II) Previous studies by the same team had shown that in a female mouse model of AD, genetic disruption of *aromatase* raised testosterone levels and lowered estrogen levels compared with what was seen in wild-type controls. Female mice missing one copy of *aromatase* had higher levels of β -site APP-cleaving enzyme 1 (BACE1) and β -amyloid ($A\beta$) and worse AD pathology than female AD mice with wild-type *aromatase*. However, because both androgens and estrogens are affected by lowering aromatase levels, it was unclear whether androgens alone could affect AD pathology.

McCallister *et al.* now establish that androgens alone can protect against AD in male mice. Male mice missing one copy of *aromatase* had higher levels of testosterone and no estrogen in the brain compared with male AD mice with wild-type *aromatase*. Unlike in females, reduced aromatase activity led to improved AD-related endpoints, including lower BACE1 and $A\beta$ levels and better memory.

Ligand Pharmaceuticals Inc. has LGD-3303, a brain-permeant selective androgen receptor modulator (SARM), in preclinical development for musculoskeletal indications and age-related hypogonadism.

Pike told *SciBX* that Li's study suggests which hormones to pursue in future trials.

"While this doesn't preclude a protective role for estrogens, in terms of drug development and future therapeutics, we can now focus on androgens," he said.

In SARM's way

Androgens have been tested in aging men as a way to prevent muscle and bone loss. Last fall, the NIH's **National Institute on Aging** launched a trial of testosterone in elderly men that is looking at a range of physical and cognitive endpoints.

But in AD, according to Pike, "we don't know when would be the best time to start a trial. Do you begin in early middle age or later when you start to see pathology? Also, it's unclear whether applying drugs late in life would be effective if the brain is already damaged."

On the flip side, starting androgen therapy preemptively in middle-aged patients could lead to adverse cardiovascular events and feminization due to conversion of androgens into estrogens by aromatase.

The solution, suggested Gandy, is to use a selective androgen receptor modulator (SARM) that specifically targets the hippocampus and cerebral cortex—the brain areas most affected by AD. Such a compound would avoid the side effects of systemic androgen therapy and might potentially be effective in both men and women, he told

SciBX.

In 2001, a team led by Gandy showed that men undergoing anti-androgen therapy for prostate cancer had higher levels of serum $A\beta$ than healthy controls.⁶

Gandy suspects that a SARM that sufficiently targets the brain could be effective both in men and women, potentially avoiding the risks of estrogen therapy.

"If you could find a way to modulate the pathway that doesn't cause the peripheral complications of hormonal manipulation, you wouldn't necessarily predict that it's only useful in males," said Gandy.

One potential candidate is LGD-3303, a SARM from Ligand Pharmaceuticals that is in preclinical development for age-related muscle and bone loss and sexual dysfunction. This month, the biotech reported that LGD-3303 penetrated the brains of female rats and stimulated sexual behavior compared with

mock treatment.⁷

"We know that the compound gets into the brain, is bioavailable and manifests an androgen-mediated effect," said Martin Meglasson, VP of discovery research at Ligand Pharmaceuticals.

Meglasson said physicians already prescribe testosterone off-label to manage anxiety and low-level depression in elderly men. Thus, testing a SARM that targets the brain, such as LGD-3303, could be a reasonable next step for clinicians.

If Li or others could demonstrate a protective effect for LGD-3303

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—Christian Pike,
University of Southern California

in a mouse model of AD, said Meglasson, “it could make a rationale for trying this SARM in men with minor cognitive impairment,” a clinical condition that often precedes AD.

He noted that LGD-3303 was designed to substantially diminish the masculinizing effects of natural androgens so that SARM therapy would not have adverse effects on sexual characteristics.

“We anticipate that our SARMs would be useful in both women and men,” said Meglasson.

Ligand’s most advanced SARM is LGD-4033, which is in Phase I testing to increase muscle strength and prevent bone fractures. That compound is not brain selective.

Other SARMs in development include Ostarine (MK-2866) from **GTx Inc.** and **Merck & Co. Inc.**, which is in Phase IIb testing for cancer cachexia, **Galapagos N.V.’s** GLPG0492, which is in Phase I testing for cachexia, and **Radius Health Inc.’s** RAD140 I, which is in Phase I for sarcopenia.

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COMPANIES AND INSTITUTIONS MENTIONED

Banner Sun Health Research Institute, Sun City, Ariz.
Galapagos N.V. (Euronext:GLPG; Pink:GLPY), Mechelen, Belgium
GTx Inc. (NASDAQ:GTXI), Memphis, Tenn.
Ligand Pharmaceuticals Inc. (NASDAQ:LGND), San Diego, Calif.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Mount Sinai School of Medicine, New York, N.Y.
National Institute on Aging, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
Radius Health Inc., Cambridge, Mass.
University of Southern California, Los Angeles, Calif.