ALZHEIMER'S DISEASE MODEL

MODEL: **AMYLOID PRECURSOR PROTEIN (APP) OVEREXPRESSING TRANSGENIC MICE, Tg2576+**

BACKGROUND AND APPLICATION:

Alzheimer's Disease (AD) causes a "triad" of cellular changes in the brain—neuronal loss, amyloid deposition, and neurofibrillary tangles. In particular, "amyloid" is a proteinaceous substance that is deposited in the brains of patients with AD. It is cleaved from a larger protein called "amyloid precursor protein," or APP. Some patients develop AD due to well-described mutations in the APP gene. One of these mutations has been incorporated into the DNA of mice—resulting in the Tg2576+ mutant mouse strain. Tg2576 mice develop amyloid deposition in the brain, and ultimately develop memory loss and dementia, much like human AD patients. Brain amyloid deposition and memory loss take about 9-12 mo. to develop in these mice.

Studies utilizing Tg2576+ mice are done for three main reasons: (1) To examine the metabolism and distribution of investigational drugs in Tg2576+ mice, (2) To examine the effects of investigational drugs on amyloid plaque deposition in the brain of Tg2576+ mice, and (3) To examine the effects of investigational drugs on modifying memory loss in Tg2576+ mice. These steps are all important parts of the pathway for developing new AD treatments that will eventually result human clinical trials, and in new marketed drugs for this disorder.

EXPERIMENTAL OUTLINE:

Tg2576+ mice, between 2-10 months old (male and female) after acclimation, are dosed with investigational agents or vehicle (as a control), at the doses, frequency, and routes specified in each individual study protocol.

Animals are observed daily for any adverse effects of treatment. At the end of each study (generally 3 months), memory testing is done using the Novel Object Recognition (NOR) test. Animals are then euthanized, and blood and tissue samples are taken for further analysis.

METHODS:

Animals

Tg2576 mice arriving at 2-10 mo. of age. Animals arrive housed in cages with their littermates (up to 5/cage). They are rearranged, so that each cage houses mice receiving the same treatment (up to 5/cage), and that treatment groups are balanced by sex and weight. Animals are acclimatized in the Vivarium for 2-12 weeks before dosing begins. Special husbandry is feeding of Lab Diet 5020 which maintains the animal weight and health.

Dosing

Dosing of test agents can be done by any route and schedule specified, including subcutaneous, intraperitoneal, intravenous, oral gavage, or intracerebral (intraventricular, intracisternal, or intraparenchymal), either by single, multiple, or continuous infusion via implanted pump.

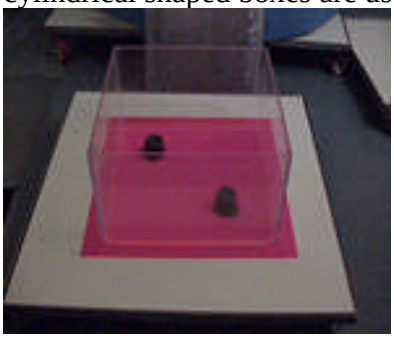
Behavioral Testing—Novel Object Recognition Test

The memory test routinely used in these studies is the "Novel Object Recognition" (NOR) test [1-3]. In this test, the animal is first familiarized with an empty box ("acclimatization period"), and is then placed in the box and exposed to two identical objects which it can freely explore ("acquisition trial"). After a variable interval (typically 2 hours), the animal is put back into the box where there remains one object from the acquisition trial ("familiar object") and a now a new unfamiliar object ("novel object"). This trial is called the "retention trial." Both acquisition and retention trials are videotaped and scored for the amount of time animals spend exploring objects. If an animal remembers the familiar object from the acquisition trial, it will spend more time exploring the novel object during the retention trial. A memory score is calculated as the time spent in exploring the novel object as a percentage of total time exploring both objects during the retention trial.

It should be noted that an important feature of the NOR test is that it depends only on the spontaneous exploratory behavior of animals. There is no obvious pain, stress, fear, anxiety, or aversive stimulation or consequences to animals used in this test.

The NOR test consists of three sessions occurring over two days. A detailed description follows:

Test apparatus: The test apparatus consists of a clear open top Plexiglas box 16x16x16 inches. The chamber is cleaned with a 1% ammonia solution between each trial. A set of rectangular and cylindrical shaped boxes are used as objects. Trials are videotaped for later scoring.



Procedure:

Session One (Acclimatization)

On Day One of NOR testing, animals are brought into the test room and are individually placed in the empty test box and allowed to explore for 5 minutes. Animals are then returned to their home cage.

Session Two (Acquisition):

Twenty-four hours after the first session, animals are brought into the test room and are placed individually in the test chamber, this time containing two identical test objects (A1 and A2) placed at opposite corners of the chamber. The animals are allowed to explore the objects for 5 minutes. The session is videotaped, and the time spent exploring each object is subsequently measured by a skilled observer. Expected normal behavior would be the animal exploring each object for an equal amount of time. Exploration is defined as the animal's nose being pointed directly at, and within 2 cm of the object. The animals are then returned to their home cage.

Session Three (Retention):

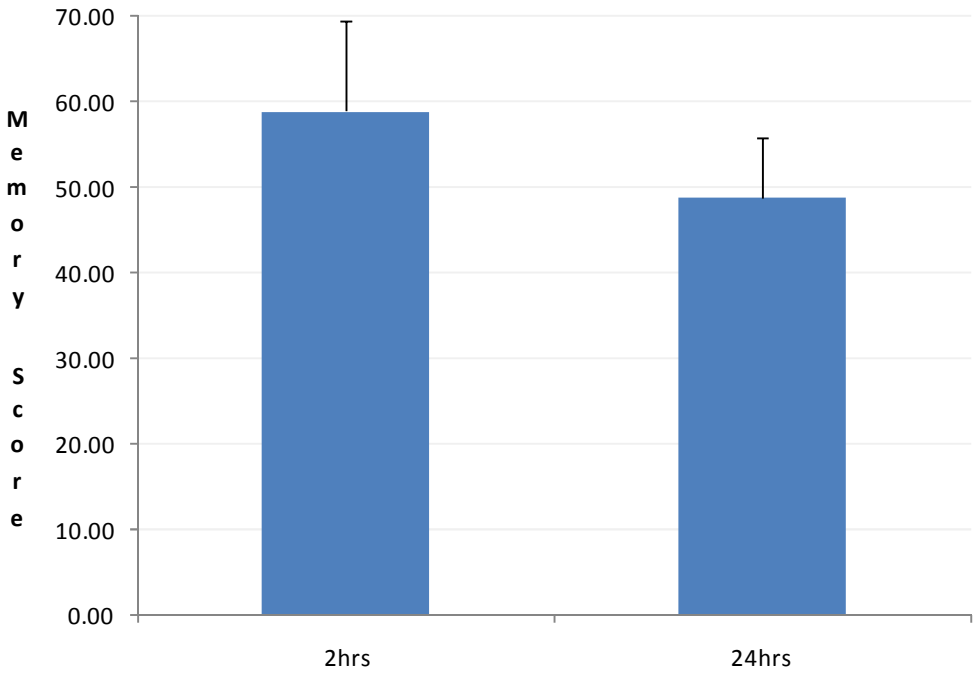
After a retention period of 2 hours, 4 hours, 24 hours or 48 hours, the animals are once again brought into the test room and are placed individually in the test chamber for exploration of 5 minutes, this time containing one object from session two and one novel object (A and B). The session is videotaped, and the time spent exploring each object is subsequently measured by an experienced observer. To assure animals are not biased toward one object, the identical objects in Session 2 are changed periodically throughout the experiment.

Expected normal behavior would be, with a short delay between Acquisition and Retention trials, that the animal explores the novel object for a longer period of time than the familiar object. Longer delay periods result in less difference between exploration time of novel to familiar object.

A "memory score is calculated for each animal, defined as the time spent in exploring the novel object as a percentage of total time exploring both objects during the retention trial. Mean memory scores between groups of animals are analyzed by standard ANOVA statistics.

Sample Data

The graph below shows typical NOR data in normal animals with a delay of either 2h or 24h between Acquisition and Retention trials. As shown, the memory score degrades during this time interval. Similar data are seen in Tg2576+ vs. wild-type mice with a 2h interval between Acquisition and Retention trials—that is, Tg2576+ mice show lower memory scores than age-matched wild-type mice.



Euthanasia/Necropsy

At the end of these studies, animals are deeply anesthetized and euthanized, and blood, CSF, brain and other organs are harvested for further analysis.

REFERENCES:

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2. Taglialatela, G., D. Hogan, W.R. Zhang, and K.T. Dineley, *Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition.* Behav. Brain Res., 2009, Epub ahead of print, Jan. 8, 2009.
3. Mouri, A., Y. Noda, H. Hara, H. Mizoguchi, T. Tabira, and T. Nabeshima, *Oral vaccination with a viral vector containing Abeta cDNA attenuates age-related Abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model.* FASEB J., 2007. **21**: p. 2135-2148.