

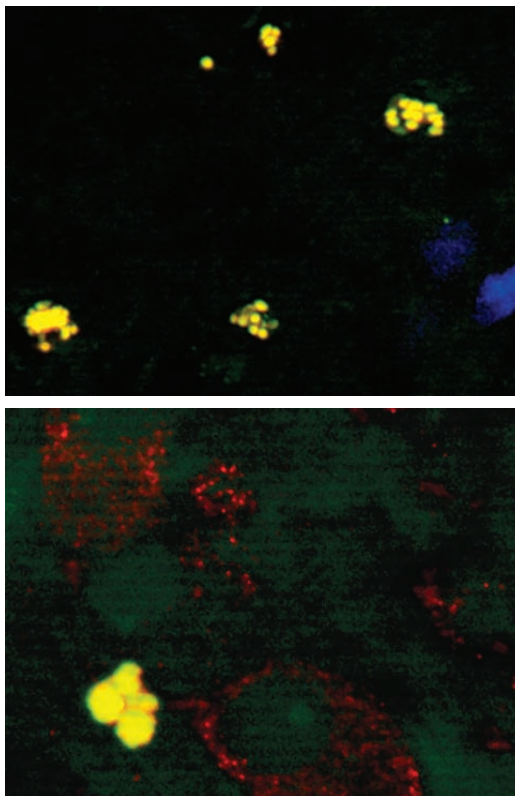
The Retina Provides a Window to the Brain for Early Alzheimer's Diagnosis

Maya Koronyo-Hamaoui, PhD

For physicians looking to diagnose Alzheimer's disease (AD) at the earliest stages, the answer might be right in the eyes. An eye scan may allow early detection of AD, opening the possibility of earlier and more effective treatment intervention. A recent study at Cedars-Sinai provides the first evidence of amyloid plaques (the hallmark pathological signs of AD) in the retinas of deceased patients, including retinas of those who were probably in early stages of the disease. Moreover, in live laboratory mice genetically modified to model the human disease, similar retinal plaques were detected *in vivo* at unprecedentedly high resolution by a newly developed, noninvasive optical imaging approach. This work has generated considerable discussion among scientists, since it reveals that AD pathology is not restricted to the brain as experts previously thought.

The retina: a better target for live imaging of AD?

AD is a devastating neurodegenerative condition and the leading cause of dementia among the elderly. It is diagnosed in patients at advanced stages of the disease, and definitive diagnosis is feasible only upon autopsy. Histological examination of postmortem brain tissue reveals the presence of distinctive changes of AD: abnormal accumulation of neurotoxic amyloid- β ($A\beta$) peptide in its aggregated form ($A\beta$ plaques) and as soluble $A\beta$, as well as the presence of intracellular neurofibrillary tangles. A variety of new diagnostic approaches are currently under development. One such technique is the collection of cerebrospinal fluid (CSF) by lumbar puncture (LP) for oligomeric $A\beta$ analysis. Unfortunately, levels of CSF oligomeric $A\beta$ have a considerable overlap in range between early AD patients and the non-AD elderly population. Furthermore, even an improved LP procedure for CSF collection was reported to cause incidences of severe headache and is highly controversial in terms of safety and feasibility. Another approach is the noninvasive imaging of amyloid burden in



Images: Yosef Koronyo

Figure 1: Postmortem retinas from Alzheimer's disease patients. $A\beta$ plaques (in yellow) identified by curcumin and anti- $A\beta$ monoclonal antibody labeling.

the brain using PET or MRI, which for now are still limited in resolution and specificity.

By contrast, the retina provides excellent possibilities for the diagnosis of AD. The retina is a developmental outgrowth of the brain and shares many similarities with the brain. It is the only organ of the central nervous system readily accessible

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for direct, noninvasive and repeated live optical imaging. Because existing noninvasive brain-imaging technologies can neither provide adequate identification nor sufficient detail regarding changes in A β plaques during early stages of AD, the Cedars-Sinai research team considered the retina as a better target for direct, high resolution live imaging of AD.

Retinal pathology in AD patients

Studies in the 1980s and '90s documented early and nonspecific visual dysfunctions in AD patients, as well as several retinal abnormalities. These abnormalities were mostly related to reduction of nerve fiber layer (NFL) thickness and loss of retinal ganglion cells in postmortem retinas of AD patients (1). Such changes are not specific to AD, however, as they appear in other eye disorders and neurodegenerative conditions, including ocular hypertension, glaucoma, multiple sclerosis and Parkinson's disease.

Recent reports demonstrated accumulation of AD-specific A β deposits in retinal samples from genetically modified mice modeling AD (2). Retinal plaques in mice were mainly detected in the NFL throughout the outer plexiform layer, and their prevalence correlated with disease progression, retinal ganglion cell degeneration, microglial activation and functional impairment. Building on this effort, the Cedars-Sinai research team turned to the examination of AD-specific deposits in postmortem retinas of human AD patients. The team was the first to discover the hallmark A β plaque pathology in retinas from AD patients (Fig. 1), which did not appear in the retinas from non-AD controls of similar age. Furthermore, retinal plaque burden appears to correlate with clinical diagnosis and brain pathology. These findings emphasized the need to develop a method of visualizing retinal plaques, a specific diagnostic marker for AD, in live subjects.

The role of curcumin labeling

The Cedars-Sinai research team found that curcumin (diferuloylmethane), a natural and safe compound of the Indian spice turmeric, is a useful agent for imaging retinal plaques. Curcumin demonstrated an ability to cross the blood-brain barrier and binds tightly to brain plaques (3). Likewise, curcumin crosses the blood-retina barrier and binds to retinal plaques. When injected to the peripheral blood of mice or given orally, curcumin enabled selective retinal plaque detection *in vivo*. By adapting an advanced microscope designed to examine live rodents' retinas, the team was able to demonstrate noninvasive visualization of individual plaques at an unprecedentedly high resolution and sensitivity.

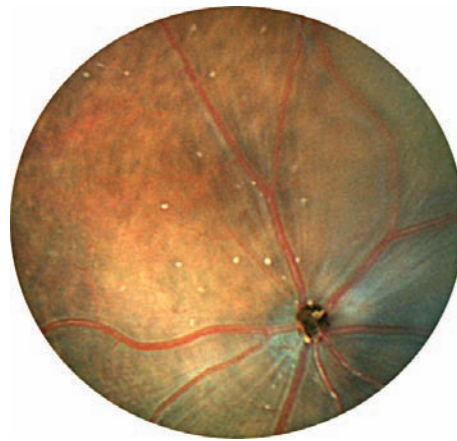


Figure 2: Noninvasive high resolution imaging of A β plaques (bright white spots) in the retina of live Alzheimer's transgenic mouse. Images captured using Micron III retinal imaging microscope.

Retinal A β plaques as an early biomarker

In murine models of AD, the research team found that A β plaques emerged in the retina months prior to their appearance in the brain and at a very early presymptomatic stage. In postmortem retinas from AD patients, plaques were found not only in the retinas of individuals with definitive AD diagnosis, but also in the retinas of those who were suspected of having early-stage AD.

Retinal plaque location and morphology were documented and compared with those found in the brain. The morphology of retinal plaques suggests that A β plaques may actually appear in the retina first. A β plaques with early-stage morphology (condensed, hard cores containing aggregated A β and the absence of radiating fibril arms, not often found in the brain) were frequently identified in retinas from AD patients.

The successful detection of retinal A β plaques in patients suspected of having AD is significant for the future of AD diagnosis and treatment. Other researchers have shown a significantly high rate of transformation of patients with mild dementia and/or mild cognitive impairment to full AD (4). Moreover, our study identified A β plaques in the retina of a cognitively normal individual who also had sparsely diffused plaques in the hippocampus, which was discovered upon brain autopsy. Further investigation of retinal plaques in larger numbers of early patients is warranted to determine their time of appearance in the retina and to quantitatively correlate AD-specific pathology in the brain to the retina.

Immunotherapy reduces retinal A β plaques

Although AD currently has no effective cure, promising therapies to slow progression are being explored. Accumulating research

suggests that AD pathology starts very early during the prodromal phase and may occur decades before clinical manifestation of cognitive impairments. Detecting AD before symptoms emerge is crucial for the testing of treatment interventions that might prevent or slow mental decline if initiated at a sufficiently early stage of the disease.

One such treatment in preclinical studies developed by our group is an immune-modulating therapy found effective in arresting cognitive decline and brain pathology in AD mouse models. A vaccination administered to AD mice recruited monocytes from the blood into the brain, and these monocytes were directly involved in A β plaque clearance and dampening of the local pro-inflammatory responses (5). As a result, a significant reduction in A β plaque burden in both retinas and brains was observed (6). These findings provide further evidence for a correlation between retinal and brain plaques and suggest similar immune mechanisms of repair.

Conclusion

Future studies are needed to demonstrate the ability of optical imaging to detect A β plaques in the retinas of living AD patients, especially in early stages of disease. If this ability is proven, imaging of retinal A β plaques offers the possibility of early, noninvasive and definitive diagnosis of AD. It sets the stage for proof-of-concept studies to validate retinal plaques as an early biomarker for AD. Most importantly, it provides what could be a powerful new tool to help evaluate early and more effective therapeutic approaches for Alzheimer's disease in human patients.

References

1. Hinton *et al*, *N Engl J Med* 1986; Blanks *et al*, *Neurobiol Aging* 1996.
2. Ning *et al*, *Invest Ophthalmol Vis Sci* 2008; Perez *et al*, *Invest Ophthalmol Vis Sci* 2009; Koronyo-Hamaoui *et al*, *NeuroImage* 2010.
3. Yang *et al*, *J Biol Chem* 2005.
4. Morris and Price, *J Mol Neurosci* 2001; Petersen *et al*, *Arch Neurol* 1999.
5. Butovsky and Koronyo-Hamaoui *et al*, *Proc Natl Acad Sci* 2006 and *Eur J Neurosci* 2007; Koronyo-Hamaoui *et al*, *J Neurochemistry* 2009.
6. Koronyo-Hamaoui *et al*, *NeuroImage* 2010.



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Translational Research and Neuroprotective Strategies to Treat Stroke

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Alteplase (tissue plasminogen activator, or tPA) is currently the only FDA-approved treatment that can be given to acute ischemic stroke (AIS) patients if they present within three hours of an ischemic stroke. After 14 years of alteplase clinical research, evidence from the European Cooperative Acute Stroke Study (ECASS) II now suggests that the therapeutic treatment window can be expanded to 4.5 hours, although this has not been formally approved by the FDA (1).

Translational research using an animal embolic stroke model with a clinically relevant endpoint leads the way for the testing of new neuroprotective strategies in AIS patients (2). More than 30 years of applied stroke research shows that there are no easy fixes for ischemic brain damage, but neuroprotection using pharmacological agents or devices shows promise in early-stage clinical trials.

Near-infrared laser therapy

The strategy of using transcranial near-infrared laser therapy (TLT) with a non-excision laser device to induce photobiostimulation is based upon the hypothesis that TLT improves energy metabolism and potentially enhances cell viability. Moreover, in preclinical animal studies, TLT is safe, promotes behavioral recovery following embolic strokes when administered up to six hours after the event, and produces a durable effect (3). In the NeuroThera Effectiveness and Safety Trial (NEST) 2, TLT was shown to be effective in AIS patients with a National Institute of Health Stroke Scale score of less than 16 and a mean time-to-treatment of 14.6 hours, significantly longer than the therapeutic window for alteplase (4, 5). The final, definitive NEST trial is pending FDA approval for initiation.

Injectable and infusion Radicut®

Radicut is a multi-target free radical scavenger approved and marketed in Japan to treat AIS patients presenting within 24 hours of the attack (6). Injectable Radicut ampoules (30 mg administered twice a day for 14 days) were first approved on May 23, 2001, and on January 19, 2010, the 30 mg

Radicut Bag for IV infusion was approved for use by the Japanese Ministry of Health and Welfare. When administered six to 72 hours following an ischemic stroke, the efficacy of Radicut ranges from large significant clinical improvements to only modest improvements in clinical function measured using standard stroke scales. In a preclinical animal embolic stroke model, Radicut administered subcutaneously decreased behavioral deficits when given up to three hours post-embolization. Moreover, the study showed that Radicut could be administered in combination with standard dose intravenous alteplase therapy (7).

In a single randomized clinical trial and numerous retrospective studies of Radicut, all research shows Radicut has a significant effect on clinical, functional or survival parameters of stroke patients, independent of the time-to-treatment and type of stroke. Based upon the available clinical data, Radicut is most effective when administered within 24 hours following a stroke. Radicut is currently being studied for safety and pharmacokinetics in AIS patients in a Phase IIa multi-center, randomized, double-blind, placebo-controlled clinical study.

Statins for stroke prevention and recovery

Another novel method to promote neuroprotection is by using statins, such as 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, which were initially designed as cholesterol-lowering drugs for the primary and secondary prevention of coronary artery disease and AIS. More recently, the pleiotropic biological activities of statins have been recognized, and many preclinical stroke studies support the testing of statins in AIS patients. Recently, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial studied the effects of atorvastatin (80 mg per day) in patients with a history of recent AIS or transient ischemic attack. The trial showed that atorvastatin reduced the overall incidence of strokes and cardiovascular events, but caused an increase in hemorrhage (8), which was observed in patients with JNC 7 Stage 2 hypertension.

The Neuroprotection with Statin Therapy for Acute Recovery Trial (NeuSTART) was designed to test the hypothesis that high-dose statins (1 to 8 mg/kg/day) administered within 24 hours of an AIS can promote clinical recovery (9,10). The initial study showed that high-dose lovastatin (8 mg/kg/day) could be tolerated for three days without adverse effects. While a non-randomized trial is ongoing, a randomized, double-blind, placebo-controlled clinical study is required to determine statin efficacy to treat AIS.

With the recent failure of numerous neuroprotective and thrombolytic strategies due to inadequate preclinical testing or toxicity, an emphasis should be placed on effective translational research studies to ultimately provide the best treatment options to AIS patients.

References

1. Hacke W *et al*, *N Engl J Med* 2008 Sep 25;359(13):1317-29.
2. Lapchak PA, *Translational Stroke Research* 2010;1(2):96-107.
3. Lapchak PA *et al*, *SPIE Proceedings* 2010;7552:75520R-R-13
4. Lampl Y *et al*, *Stroke* 2007 Jun;38(6):1843-9.
5. Zivin JA *et al*, *Stroke* 2009 Apr;40(4):1359-64.
6. Lapchak PA, *Expert Opinion on Pharmacotherapy* 2010;11(10):1753-63.
7. Lapchak PA, Zivin JA, *Exp Neurol* 2009 Jan;215(1):95-100.
8. Amarenco P *et al*, *N Engl J Med* 2006 Aug 10;355(6):549-59.
9. Elkind MS *et al*, *Int J Stroke* 2008 Aug;3(3):210-8.
10. Elkind MS *et al*, *Cerebrovasc Dis* 2009; 28(3):266-75.



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Los Angeles County: Speeding Stroke Patients to Effective Therapeutic Intervention

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When local and regional government entities adopt the concept of ambulance-directed stroke transport, area residents receive previously unprecedented access to early intervention as well as comprehensive care for especially complicated cases. Although a few networks have existed for more than a decade, the majority began to gel when the Joint Commission started certifying Primary Stroke Centers a few years ago. Efforts by the Brain Attack Coalition, the American Stroke Association and other organizations encouraging aggressive intervention played a significant role.

Los Angeles County's Emergency Medical Services (EMS) Agency instituted its stroke response plan last November. Patients suspected of suffering acute strokes are now transported directly to Approved Stroke Centers that are committed to being prepared 24 hours a day—because while time to treatment is important, the type of treatment is critical. As many as one-third of all patients with stroke come to an emergency department within the appropriate time window to receive intravenous thrombolytic therapy. A much smaller percentage, however, actually receive this life- and ability-sparing intervention.

Cedars-Sinai was among the first nine sites identified in Los Angeles County as Approved Stroke Centers, and we have seen an increase in the number of EMS-transported patients. We currently evaluate 300 to 400 patients per year for acute stroke, treating two to seven patients a month. Many patients now receive treatment that would not have been possible before the county's emergency stroke system was activated.

The recent experience of a healthy, active woman in her mid-60s illustrates the community benefits of ambulance-directed acute stroke care. She was leading an exercise class at a gym one morning when she collapsed, exhibiting right-side facial droop, right arm paralysis and aphasia.

Responding paramedics—who previously would have taken the patient to the closest hospital, located just blocks from the gym—redirected to Cedars-Sinai, the nearest Approved Stroke Center. They were met at the Emergency Department by members of our “Code Brain” team, which includes neurologists and nurses specializing in stroke, as well as pharmacists.

The patient, who had no previously known stroke risk factors, evaluated at 21 on the NIH Stroke Scale, suggestive of a severe stroke. Computed tomography (CT) without contrast and CT perfusion studies showed no bleed but distal occlusion of the middle cerebral artery, with decreased blood volume anteriorly and a mismatched portion of decreased perfusion.

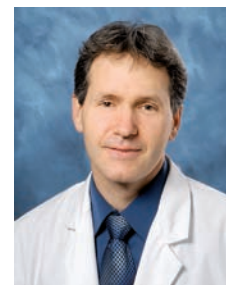
Within about two hours of symptom onset, IV tPA was administered, as was the anti-coagulant argatroban, offered as part of a six-center clinical trial investigating this combination therapy. This is one of several acute stroke studies currently underway.

Without direct transport to a specialized stroke center and early intervention, odds would have been stacked against a favorable outcome. Instead, the patient was hospitalized in Cedars-Sinai's specialized neuro-critical care unit for 11 days before being transferred to inpatient rehabilitation. She went home 10 days later with only mild deficits, minimal need of assistance, and what appears to be a much better outlook for the future.

The Approved Stroke Center system, in addition to speeding stroke patients to rapid-response care, is designed to improve patient access to the appropriate level of care. The system does not require that every Approved Stroke Center have every capability or even have a neurosurgeon available at all times. It does require that a neurosurgeon be available within two hours of a patient's arrival, and for smaller centers, this can be accomplished by transferring the patient to a nearby center where a neurosurgeon is standing by.

To meet the criteria, smaller Approved Stroke Centers enter into agreements to immediately transfer certain patients to tertiary centers that offer 24-hour rapid-response teams, 24-hour neurosurgical coverage, neurointerventional services with thrombectomy devices, and specialized neuro-critical care units and stroke/neuro nursing units. If, for example, a patient arriving at a smaller stroke center is not a candidate for IV tPA, is outside the treatment window, or fails to respond to treatment as the physician expects, transfer to a more comprehensive center is initiated. Rapid access to neuro-endovascular intervention, such as revascularization devices and experimental stents, are among the tertiary services now available.

While Los Angeles county is not the first metropolitan area to implement such a system, the benefit to stroke victims is not to be minimized. We strongly encourage our colleagues in other cities to advocate for ambulance transport guidelines and tertiary stroke care networks that quickly funnel patients to Joint Commission-certified Primary Stroke Centers.



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