Drug Discovery Today: Technologies
Vol. 8, No. 2–4 2011
Editors-in-Chief
Kelvin Lam – Blue Sky Biotech Inc., USA
Henk Timmerman – Vrije Universiteit, The Netherlands
Imaging techniques

Pre-clinical optical imaging and MRI for drug development in Alzheimer's disease

Maarten Rotman[1,2,*], Thomas J.A. Snoeks[3], Louise van der Weerd[1,2]

[1]Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
[3]Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands

Optical and magnetic resonance imaging have the potential to be complementary non-invasive imaging modalities. Yet without advances in imaging technologies and contrast agents both have short-comings that cannot be ignored. In this review we demonstrate the pre-clinical use of the two imaging techniques in Alzheimer's disease, including examples from recent applications and discuss what is needed to improve their applicability for drug discovery.

Guest Editors:
Nicolau Beckmann – Novartis Institutes for BioMedical Research, Basel, Switzerland.
Bert Windhorst – VU Medical Center, Amsterdam, The Netherlands.

Introduction

Alzheimer's disease (AD) is a common illness with an increasing incidence as populations get older. The disease is pathologically characterized by aggregates of amyloid β peptides (Aβ) and intercellular neurofibrillary tangles of tau proteins. Currently, no cure exists for AD, but many drug candidates are being developed, focusing both on the prevention or break-down of the protein aggregates and on treatment of the cognitive symptoms.

Pre-clinical research into AD primarily uses transgenic mouse models carrying human mutations in the APP and gamma secretase components PSEN1 and PSEN2 genes, leading to accelerated amyloid plaque formation. These models allow for monitoring of disease progression and screening intervention efficacy, although they are limited to the amyloid-related aspects of the Alzheimer's pathogenesis. New triple mutation models have been developed that combine amyloid plaque formation and tau tangles [1]. These models could prove to be very useful for future drug development.

Fluorescence imaging

Optical imaging methods are sensitive methods for *in vivo* imaging, based on the detection of photons emitted from the tissue of interest. For *in vivo* fluorescence imaging (FLI), fluorescent proteins or dyes are used that need external excitation for light emission, with wavelengths in the visible and near infrared parts of the spectrum. Optical imaging techniques are characterized by their high sensitivity and specificity towards the target of interest. However, the tissue penetration of visible light is limited, and scattering and absorption of the photons by tissue impair precise localization and quantification of the signal (Table 1).

In contrast to non-invasive whole body fluorescent imaging, techniques like multi-photon microscopy can be used to image intact tissues with sub-cellular resolutions [2]. Multi-photon techniques are based on exciting a fluorophore by simultaneous absorbance of two long wavelength photons rather than one short wavelength photon, which is the case

*Corresponding author.: M. Rotman (m.rotman@lumc.nl)

 DOI: 10.1016/j.ddtec.2011.11.005

Table 1. Comparison of FLI and MRI

	Fluorescence imaging (FLI)		Magnetic resonance imaging (MRI)
	Whole body imaging	**Multi-photon**	**μMRI, MRS, MRA, fMRI**
Pros	Rel. inexpensive, straightforward imaging hardware High throughput Wide range of contrast agents available	Sub-cellular resolution Wide range of contrast agents available Quantitative	Unlimited penetration depth High anatomical resolution Possible to detect related alterations, for example, in blood flow, atrophy, metabolites Well established in clinical practice Quantitative
Cons	Limited tissue penetration Low resolution Semi-quantitative	Cranial window is highly invasive Limited field of view Time-consuming	Rel. expensive and complex imaging hardware Contrast agents not as advanced More time-consuming than whole body FLI
Refs	[6]	[2]	[12,25,28]

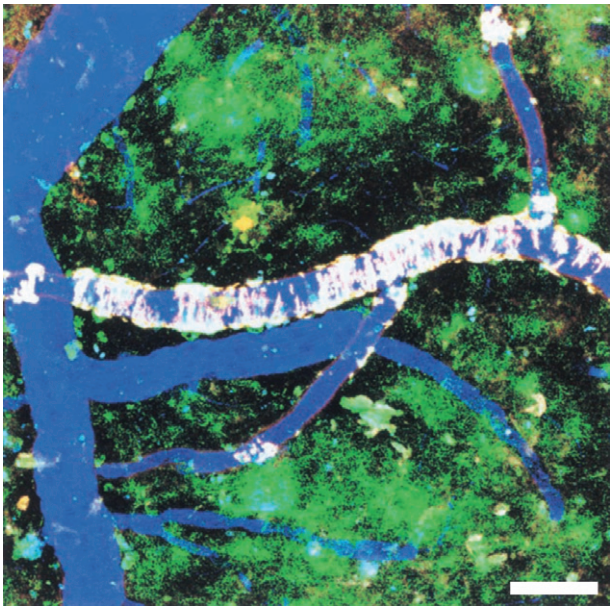
with normal fluorescence. The use of long, near infrared, wavelengths and two photons simultaneously has several advantages compared to lower wavelength photons, such as better in-depth localization, deeper tissue penetration and lower photo-toxicity [2]. This technique requires the presence of an optical window, that is, an intracranial window when the brain is studied. The placement of this window requires a time-consuming operation procedure and makes the techniques more invasive than, for instance, magnetic resonance imaging (MRI). However, the advantage of the window is that the major source of inaccuracy in optical imaging, namely absorption and scattering by tissue, is greatly reduced, thus improving the quantification of the fluorescent intensity, although the measurements are restricted to the small part of the brain that lies directly under the window.

FLI detection of amyloid depositions

Imaging of Aβ aggregates in the brain is one of the most straightforward ways to follow AD disease progression. These dense core and diffuse Aβ deposits have been imaged using Aβ-specific antibodies coupled to a fluorescent label. Examples of such antibodies are alexa-fluor-750-conjugated BAM-10 [3] and fluorescein-labeled 10d5 [2]. Most of these ligands need to be applied locally, as they do not easily cross the blood–brain barrier (BBB). Figure 1 shows an example where multi-photon microscopy was used to image Aβ deposits in the brain, Aβ deposits on blood vessels and the microvasculature simultaneously.

Nowadays, mostly small amyloid-targeting fluorescent molecules are used, the most standard one being the Congo Red derivative methoxy-X04, which readily crosses the BBB and is administered via a straightforward intraperitoneal injection. Another well-known compound is Thioflavine-S [3,4], which not only binds to Aβ deposits, but also to neurofibrillary tangles.

These techniques have been used to study the beneficial effects of natural compounds like curcumin, an Indian spice with a broad spectrum of anti-oxidant, anti-inflammatory and anti-fibrilogenic activities. *In vivo* multiphoton microscopy showed that curcumin crossed the blood–brain barrier, and after systemic treatment of mice for seven days, curcumin cleared and reduced existing plaques. Curcumin labels amyloid pathology *in vivo*, disrupts existing plaques, and



Drug Discovery Today: Technologies

Figure 1. Multicolor fluorescence from the cortex of a PDAPP mouse. This composite image represents a three-color projection of a three-dimensional volume of the cortex of a living transgenic mouse. Thioflavine-S, displayed in red, labels dense-core amyloid deposits on blood vessels (*e.g.* traversing horizontally in the middle of the image) and plaques (yellow deposit, just above center). Fluorescein-labeled 10d5 antibody, displayed in green, labels dense-core and diffuse amyloid deposits in the brain. Texas red dextran (70 kDa), displayed in blue, was injected into a lateral tail vein to allow fluorescence angiography. This dye does not cross the blood–brain barrier and fills all blood vessels. All three dyes were excited simultaneously at 750 nm, and fluorescence emission was captured with a three-channel external detector system. The projected volume is about 150 mm deep from the surface of the cortex. The scale bar is 100 mm (Reprinted with permission Fig. 2 from Bacskai *et al.* [2]).

partially restores distorted neurites in an APPswe/PS1dE9 Alzheimer's disease mouse model [5].

Fluorescent probes like AOI987 and the curcumin-derivative CRANAD-2 are more recent additions to the arsenal of Aβ-targeting probes [6,7]. The fluorescence intensity, excitation/emission wavelengths and fluorescent lifetime of these probes change upon binding to Aβ [6,8]. These changes make it possible to selectively image the Aβ-bound probe, minimizing a-specific background signal due to the unbound probe present in the tissue. These properties are particularly useful for non-invasive whole-body FLI studies, where the resolution is not sufficient to detect the actual plaques.

Lastly, the recent development of transgenic mice that express luciferase under control of the Gfap promoter as well as mutant human amyloid precursor protein (Tg APP23:*Gfap*-luc) allows monitoring of Aβ accumulation without the need for an external fluorophore or light source (Fig. 2) [9].

FLI detection of structural alterations

Apart from direct detection of amyloid aggregates, optical imaging is a suitable modality to study the interaction between plaques and healthy brain tissue. Using fluorescein-labeled tomato lectin, Bacskai *et al*. imaged microglial cells surrounding dense plaques, up to a depth of about

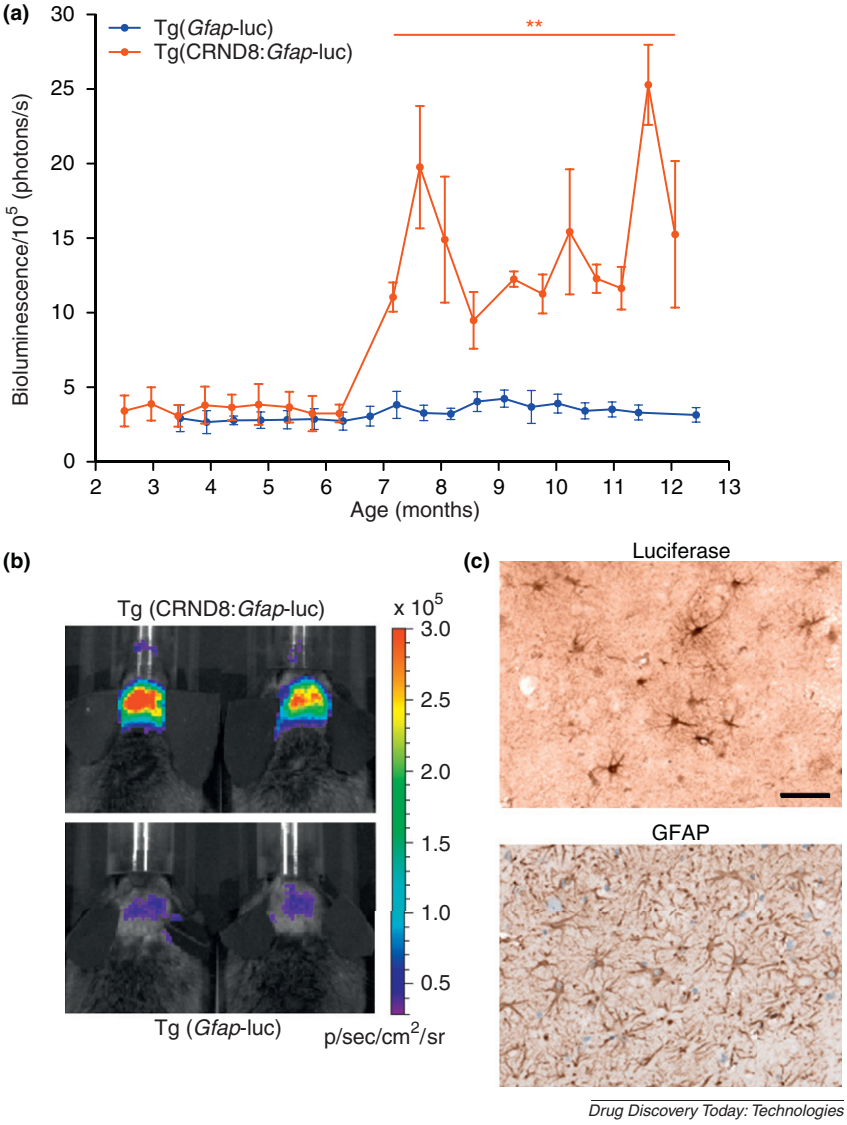


Figure 2. Bioluminescence imaging of disease progression in Tg(CRND8:Gfap-luc) mice. **(a)** Mean bioluminescence (±SEM) signals obtained from Tg(CRND8:Gfap-luc) mice (red; $n = 5$) were compared with Tg(Gfap-luc) mice (blue; $n = 8$) at 2.5–12.5 months of age. Whereas Tg(Gfap-luc) mice did not exhibit an increase in the BLI signal with age, Tg(CRND8:Gfap-luc) mice showed a statistically significant increase in bioluminescence beginning at 7 months of age, which continued for the duration of the experiment (**$P < 0.01$ at each time point). **(b)** BLI signals from the brains of 12-month-old Tg(CRND8:Gfap-luc) mice (upper) are prominent compared with age-matched Tg(Gfap-luc) controls (lower). **(c)** Immunohistochemistry on the brain of a 12-month-old Tg(CRND8:Gfap-luc) mouse shows prominent luciferase (upper) and GFAP (lower) staining in astrocytes within the hippocampus (scale bar: 50 μm) (Reprinted with permission Fig. 2 from [9]).

50 μm from the surface of the brain of living Tg mice that overexpress human APP [2]. In another study, D'Amore *et al.* looked at the structural changes of neuronal cells in close proximity to plaques in PDAPP mice. Diffuse amyloid and dense core plaques were labeled *in vivo* with fluorescein-conjugated antibodies and Thioflavine-S, and a set of neurons and neurites was labeled using dextran-lysine conjugated Alexa Fluor 594. Multi-photon imaging revealed structural changes such as increased curvature and bulbous outgrowths in neuronal processes near Aβ deposits [10]. As an example of the use of these techniques in drug research, Spires-Jones *et al.* recently published a paper detailing the acute effect of passive immunotherapy on dendritic spines. Within one hour of applying a neutralizing anti-Aβ antibody, they showed a small but significant increase in dendritic spine formation in PDAPP mice brain far from plaques, demonstrating that removing toxic Aβ species rapidly increases structural plasticity [11].

Magnetic resonance imaging

In contrast to optical imaging, MRI is characterized by its high anatomical resolution and intrinsic contrast, combined with virtually unlimited tissue penetration. As such it is one of the most frequently used imaging modalities in pre-clinical, and clinical, *in vivo* characterization of AD. Analogous to FLI, MRI can be used to image Aβ depositions in the murine brain, but is in addition very well suited to monitor other AD-related morphological and functional alterations.

MRI detection of amyloid depositions

Amyloid beta plaques of over 100 μm in diameter can be directly detected by MRI as hypointense spots in high-resolution T2 or T2* weighted images [12]. Figure 3 shows how circular hypointense spots on MRI co-localize with *ex vivo* immunohistochemistry staining against Aβ depositions. It has been postulated that the MRI hypointensities are the direct result of iron deposition inside the dense core plaques. Iron is indeed found in many senile plaques, and increasing iron content in the brain, along with other metals, correlates with the increase in beta amyloid burden [13]. However, iron does not deposit uniformly in individual plaques, nor does it deposit in all plaques present in the murine brain. Furthermore, the deposition of metals in the individual plaques does not occur until the disease has progressed into late stage, and is not found in younger animals [13,14]. On top of that,

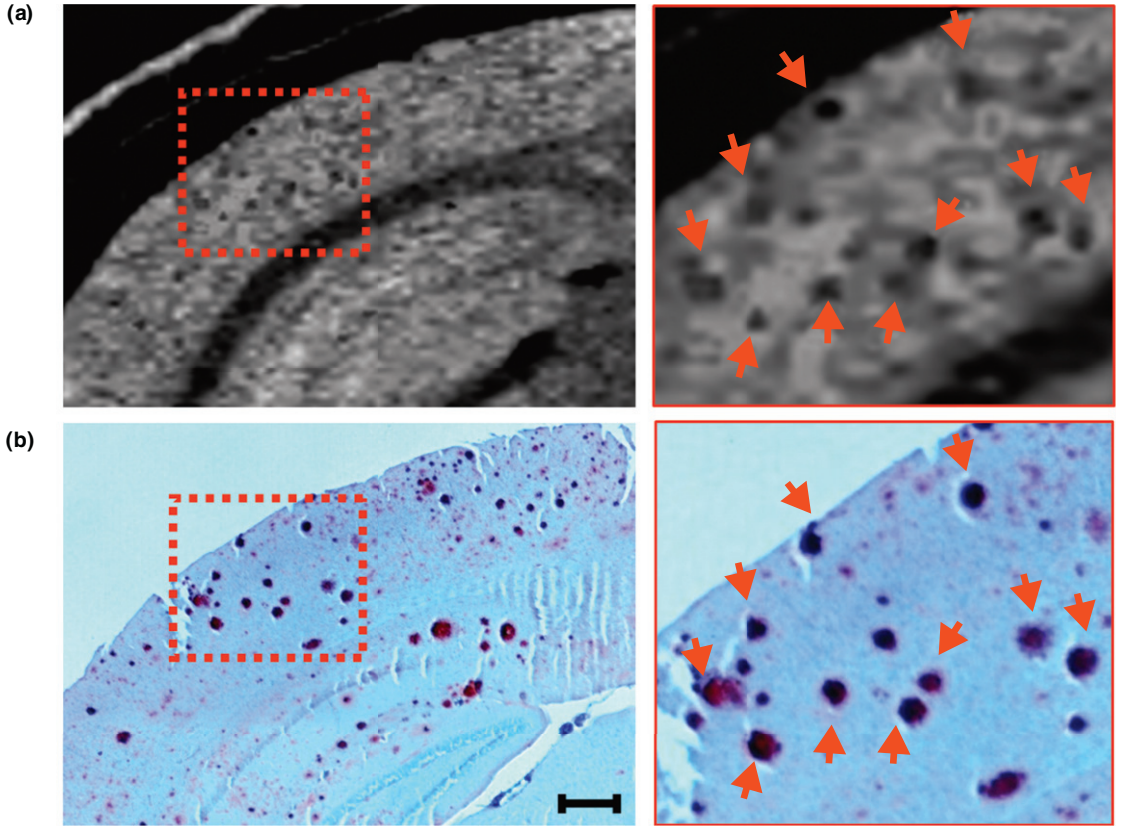


Figure 3. Coregistration of Aβ plaques seen in the brain of an 18-month-old AD mouse by **(a)** *in vivo* μMRI and **(b)** histological section of the same mouse brain stain with Aβ antibodies. Many MR circular hypointense spots can be matched to immunostained Aβ plaques (arrows), which can be seen more clearly in the higher-magnification insets. Scale bar: 500 μm (Reprinted with permission Fig. 2 from Braakman *et al.* [12]).

T2/T2* weighted hypointensities are not limited to AD and most amyloid plaques are smaller than 100 μm, which all may lead to misidentification of the observations [15]. Finally, as a consequence of the generally small amyloid plaque size, the long scan times that are often necessary to achieve acceptable resolution may make the procedure challenging for routine application in *in vivo* pre-clinical studies [16].

As a result, attempts have been made to facilitate a reliable *in vivo* detection of individual Aβ depositions by the use of specific MRI contrast agents. Such contrast agents can be gadolinium chelates or superparamagnetic iron oxide nanoparticles tagged to amyloid peptides or to anti-Aβ monoclonal antibody fragments [17–19] or even untargeted gadolinium [20]. Also FSB, a fluorinated amyloidophilic compound closely related to the optical imaging agent methoxy-XO4, and detectable by ^{1}H and ^{19}F MRI has been reported to target plaques in 12- to 24-month-old APP Tg mice [21].

However, up to now all of the above mentioned Aβ-specific contrast agents rely on the co-injection of mannitol or DMSO to artificially open the blood–brain barrier (BBB), or employ intra-cranial injection of the agent, effectively reducing the non-invasive character of MRI and limiting longitudinal studies.

As an alternative to monitoring individual plaques, it is possible to quantify greater regions of interest. Teipel *et al.* described an automated, voxel-based analysis of T2 relaxation time changes in the entire brain of an APP/PS1 transgenic AD mouse model. While this approach does not allow visualization of individual plaques, it is applicable at lower field strengths and can be easily quantified, without the use of any contrast agents and is not limited to *a priori* selected areas [22].

Considering the current Aβ cascade hypothesis, monitoring altered Aβ deposition buildup over time may be the most direct and specific method for the effect of anti-AD drug intervention, and drug development as such. However, the strength of MRI is that it is also applicable for measurements of the consequences of amyloid aggregates on brain structure and function.

Detection of brain atrophy

In clinical studies and diagnosis of AD, the occurrence of cerebral atrophy is well characterized and volumetric assessments are used routinely on patients with mild cognitive impairment (MCI) and AD. By contrast, brain atrophy in AD mouse models is more difficult to detect and subject to several challenges, among which the fact that the murine brain is approximately a 3000-fold smaller than the human counterpart and the high contrast between gray and white matter in the human brain is absent in mice [23]. But in an APPswe/ind AD mouse model, Badea *et al.* demonstrated a proof-of-principle that, as in human MCI and AD patients, amyloid deposition is preceded by MRI detectable cortical volume loss [24]. With the development of new tauopathy murine models, in which brain atrophy seems more predominant, and continued development of scan and analysis techniques and protocols, volumetric MRI may become an efficient translational imaging tool in pre-clinical AD drug development [25].

Detection of microvascular alterations

Much effort has been put into imaging the functional consequences of amyloid deposits.

One such approach employs the increased presence of macrophages and microglia around vessels heavily affected with cerebral amyloid angiopathy (CAA), the vascular deposition of Aβ [26]. Untargeted iron oxide nanoparticles are being taken up by the macrophages through absorptive endocytosis and subsequently provide enhanced contrast, allowing non-invasive monitoring of the development of vascular pathology, correlated with Aβ build-up. Using this method, in combination with histological *post mortem* analysis, Beckmann *et al.* showed how prolonged passive Aβ immunotherapy treatment increased the incidence of vascular lesions co-localizing with iron-loaded macrophages in APP23 mice that develop CAA.

Also the increase of BBB permeability can be detected on MRI. Contrast enhanced T1-weighted images shows leakage of the contrast agent gadolinium-DTPA into tissue surrounding CAA-affected vessels [27]. By increasing the phagocytosis of amyloid by macrophages, Lifshitz *et al.* have been able to reduce the leakage in a TGF-β1 transgenic mouse model back to WT control levels and thereby demonstrated the value of this method in drug development.

A clinically well-known feature of CAA is the occurrence of microhemorrhages. These microbleeds are also present in some transgenic AD models, and can be detected using T2*-weighted scans without the use of contrast agents [28]. Susceptibility-weighted MRI is applied in clinics to detect microhemorrhages in AD patients and as such, this technique, applied in murine models, is supplied with a potentially well-established translational platform. Microhemorrhage susceptibility is a risk factor for vasogenic edema, correlated with Aβ immunotherapy in (pre-)clinical trials and the ability to monitor their incidence may be crucial for successful therapy development [28].

On a microvascular level, MRI measurements of cerebral perfusion monitor the delivery of arterial blood to the capillary bed. As such it has been found that perfusion and cerebral blood volume are decreased in transgenic models of AD [29]. Mechanistically this could lead to a state of hypoperfusion, and thereby exacerbate the cognitive symptoms. Cerebral perfusion measurements have, for example, been applied to determine the effect of dietary lipids on brain circulation in APP/PS1 Tg-mice, showing how increased intake of dietary

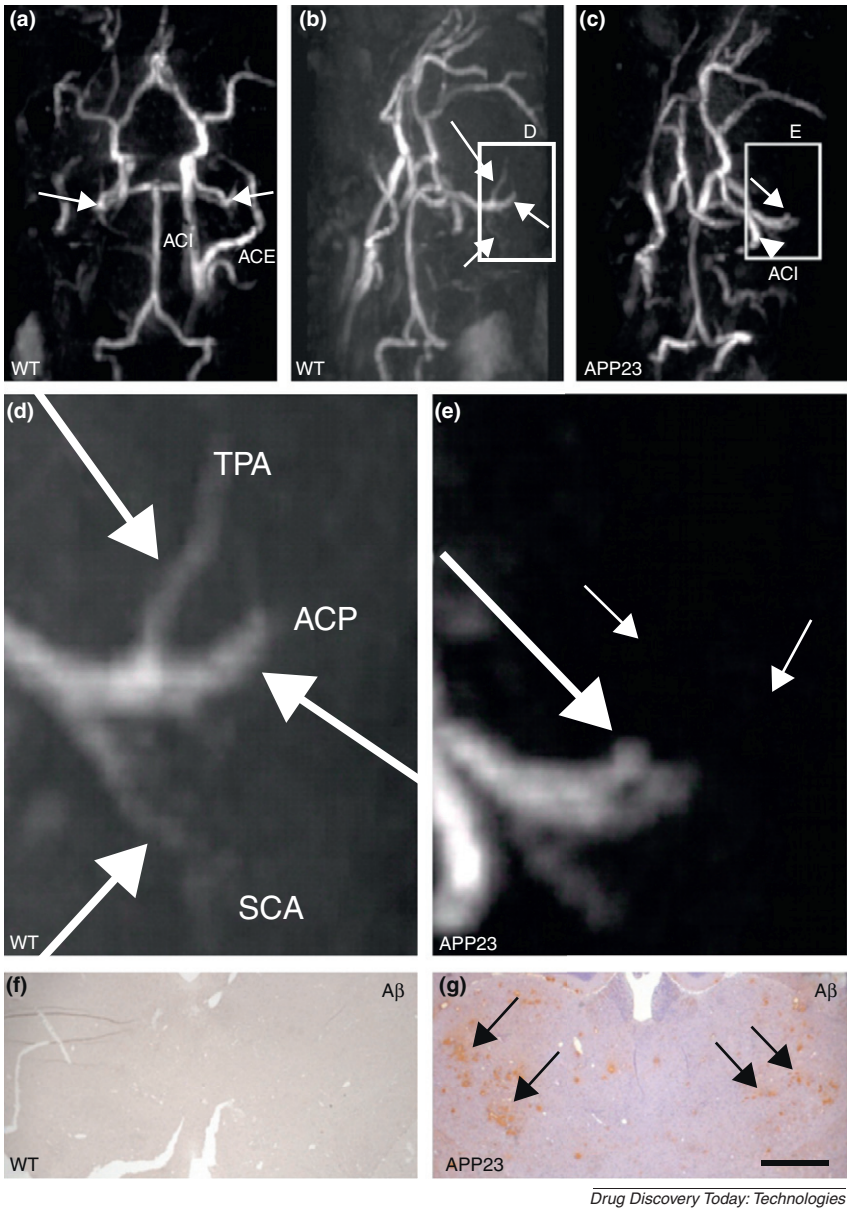


Figure 4. MRA images of a 26-month-old, male wild-type mouse without flow disturbances (**a**: anterior–posterior projection; **b** and **d**: oblique projection) and a 26-month-old, male APP23 mouse with evident alterations of cerebral blood flow (**c** and **e**: oblique projection). (a, b and d) All vessels in the wild-type mouse exhibited a conspicuous blood flow signal (arrows in a, b and d). There was no evidence for stenosis or occlusion of the vessels. The posterior cerebral artery branches into the superior cerebellar artery (SCA), the thalamoperforating artery (TPA) and the main part of the posterior cerebral artery (ACP) best seen in an oblique projection (b and d). In the APP23 mouse the TPA and the main part of the ACP were not detected distal to the trifurcation (large arrow in c and e) indicating disturbances in cerebral blood flow. The small arrows in (e) point to the area where TPA and ACP were seen in the non-transgenic mouse (Reprinted with permission Fig. 5 from Thal *et al.* [32]).

docosahexaemoic acid, or DHA, is likely to increase relative cerebral blood volume but not blood flow [30].

On a somewhat larger scale, blood flow analysis or magnetic resonance angiography (MRA) can be employed to follow the alterations of bigger blood vessels in transgenic mouse models. The inflow of water protons inside the vessels is used to generate high contrast with respect to residual signal from saturated protons in surrounding stationary tissue, a technique known as time of flight (TOF-) angiography. A drawback of TOF-angiography is that the smaller veins and arterioles remain practically undetected due to low flow rates. In contrast-enhanced (CE-) angiograms, the use of a paramagnetic contrast agent in the bloodstream allows higher contrast and more detail, up to the level of the veins, and may allow visualization of blood vessels which are not detected by TOF-MRA [31]. Using MRA, Thal *et al.*

demonstrated how wild-type mice did not show the blood flow disturbances that were found in their APP23 transgenic model, as shown in Fig. 4 [32].

Detection of functional alterations

In addition to monitoring buildup of Aβ and alterations in the cerebral vasculature, it is also possible to use MRI to look at the functional consequences of progressive AD in rodent models and the effects of experimental treatment.

One such approach recognizes the fact that in triple transgenic mice, axonal transport impairment has been linked to the pathogenic processes of the disease. Manganese-enhanced MRI (MEMRI) utilizes the fact that the manganese ion (Mn^{2+}) is a paramagnetic calcium analog, which is competitively taken up into neurons via voltage-gated calcium channels, packaged into vesicles and transported down the axon. Therefore, manganese chloride ($MnCl_2$) can be used as a contrast agent to map the overall axonal transport integrity [33]. For example, it has been used to demonstrate the positive effect of the Aβ42 selective lowering agent R-flurbiprofen on axonal transport in the Tg2576 ($SweAPP695_{[K670N/M671L]}$) mouse model. In this study, the authors show how reductions in both Aβ42 level- and tau hyperphosphorylation as a result of chronic R-flurbiprofen treatment correlate with improved transport as assessed by MEMRI [34].

Metabolic changes can be assessed using ^1H MR Spectroscopy (MRS). Using MRS, metabolites can act as functional markers, for example, lactate for impaired metabolism and cerebral blood flow, *N*-acetylaspartate for neuronal number, integrity and health and thus for neurodegeneration, *myo*-inositol for glial cells and cerebral osmolarity, although absolute quantification is challenging [35]. Using MRS it has been determined that in an inducible Tau transgenic model signs of gliosis, that is, increased *myo*-inositol, were indeed increased compared to wild-type [25].

Finally, a widely used approach in clinical cases of neurodegenerative disorders is functional MRI or fMRI. This very interesting tool still has many technical challenges to overcome for useful pre-clinical application, including the limited knowledge on the effects of anesthetic agents and the lack of relevant challenging paradigms. To partly circumvent these issues, it is possible to trigger brain activation by electrically or pharmacologically stimulating brain regions of interest, while simultaneously monitoring brain activation, for example, by using MEMRI, blood-oxygen level-dependent (BOLD) fMRI or CBV-based fMRI [23,36]. For instance, by applying cerebral blood volume-based fMRI Princz-Kranz *et al.* determined the diminished response of cerebral blood vessels to the vasodilatory compound acetazolamide in aged arcAβ Tg-mice, a model that develops both parenchymal and vascular depositions of Aβ. The altered fMRI response with respect to wild-type animals may serve as a diagnostic biomarker for cerebral Aβ deposition [37].

Conclusion

Both FLI and MRI have proven to be effective in the *in vivo* detection of Aβ depositions and related alterations, but both have issues which should be addressed. FLI has excellent contrast agents, some even able to cross the blood–brain barrier independently, but lacks the penetration depth found in MRI. Attenuation of light by tissue greatly interferes with its application. Emerging transgenic bioluminescent models may improve at least some of these problems.

Alternatively, a cranial window in the mouse model certainly alleviates this problem; however, the procedure is invasive, requires a time-consuming operation procedure and limits the view to the underlying cranial region and may therefore not yet be easily applied in routine drug development research.

MRI, by contrast, requires expensive equipment and specialized personnel, even although it may still score better on these aspects than for instance PET imaging. Its intrinsic contrast can be applied to the detection of larger plaques; however, without the aid of contrast agents, confident detection of amyloid plaques in the murine brain is very challenging. Region-of-interest analyses to detect T2 changes associated with iron accumulation around plaques will probably be more time-efficient and allow for higher throughput. Contrast agents for MRI are being developed, but lack the sensitivity and the efficient BBB passage found in FLI contrast agents. Co-injection with BBB modulating agents such as mannitol is sufficient to bypass this problem, but ideally this would not be necessary.

MRI combines high resolution with good soft tissue contrast and is very well suited to detect both functional and anatomical alterations associated with AD. Moreover, MRI can examine the structure of the whole brain in any plane and generate three-dimensional images suitable for detailed volumetric quantification. Alterations in metabolites, cerebral blood flow and brain activation can be measured using MRI. These techniques are currently applied in clinical settings as well, which makes eventual translation to a clinical setting easier. The development of cost-efficient MRI systems, such as the low-field Bruker Icon^{TM} (1T) and bench-top Vision MRI, and the introduction of improved detection techniques such as the highly sensitive but rather costly cryogenic radiofrequency probes [38], will open up the MRI field to more researchers and better detection of amyloid depositions.

As shown here with examples of recent publications, currently both imaging modalities, FLI and MRI, yield studies validating the detection of Aβ depositions or related alterations, sometimes using a validated pre-clinical therapy method such as antibody treatment to strengthen the evidence. These studies have yielded novel contrast agents or imaging

procedures, but up to now not a single novel therapeutic has been introduced using these imaging modalities. This is in part due to the common acceptance that the gold standard in defining AD, and thus effect of treatment, is neuropathology [39]. Hence all statements made above were backed up by *ex vivo* immunohistochemistry, and the studies are actually proof-of-concepts of the detection method. A second problem may be that currently no common standard has been proposed for pre-clinical diagnosis of AD phenotype based on MRI or FLI. A next step towards universal pre-clinical diagnosis and monitoring of AD would be to standardize protocols and to introduce between-site validation of the imaging methods. On such a basis, drug development in search for *in vivo* diagnosis and cure of AD could be more straightforward, reproducible and, importantly, comparable.

Conflict of interest

The authors have no conflict of interest to disclose.

Acknowledgments

The authors thank Brigit den Adel, MSc. for discussion on the manuscript. This work has been supported by the Center for Medical Systems Biology (CMSB2), the Center for Translational Molecular Medicine (LeARN), IOP Genomics (IGE05005), and NWO Athena (700.58.801).

References

1 Wisniewski, T. and Sigurdsson, E.M. (2010) Murine models of Alzheimer's disease and their use in developing immunotherapies. *Biochim. Biophys. Acta* 1802, 847–859
2 Bacskai, B.J. and Hyman, B.T. (2002) Alzheimer's disease: what multiphoton microscopy teaches us. *Neuroscientist* 8, 386–390
3 Skoch, J. *et al.* (2005) Development of an optical approach for noninvasive imaging of Alzheimer's disease pathology. *J. Biomed. Opt.* 10, 11007
4 Skoch, J. *et al.* (2006) Preclinical characterization of amyloid imaging probes with multiphoton microscopy. *J. Alzheimers Dis.* 9, 401–407
5 Garcia-Alloza, M. *et al.* (2007) Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. *J. Neurochem.* 102, 1095–1104
6 Hintersteiner, M. *et al.* (2005) In vivo detection of amyloid-beta deposits by near-infrared imaging using an oxazine-derivative probe. *Nat. Biotechnol.* 23, 577–583
7 Ran, C. *et al.* (2009) Design, synthesis, and testing of difluoroboron-derivatized curcumins as near-infrared probes for in vivo detection of amyloid-beta deposits. *J. Am. Chem. Soc.* 131, 15257–15261
8 Raymond, S.B. *et al.* (2008) Smart optical probes for near-infrared fluorescence imaging of Alzheimer's disease pathology. *Eur. J. Nucl. Med. Mol. Imaging* 35 (Suppl. 1), S93–S98
9 Watts, J.C. *et al.* (2011) Bioluminescence imaging of Abeta deposition in bigenic mouse models of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 108, 2528–2533
10 D'Amore, J.D. *et al.* (2003) In vivo multiphoton imaging of a transgenic mouse model of Alzheimer disease reveals marked thioflavine-S-associated alterations in neurite trajectories. *J. Neuropathol. Exp. Neurol.* 62, 45
11 Spires-Jones, T.L. *et al.* (2009) Passive immunotherapy rapidly increases structural plasticity in a mouse model of Alzheimer disease. *Neurobiol. Dis.* 33, 213–220
12 Braakman, N. *et al.* (2006) Longitudinal assessment of Alzheimer's beta-amyloid plaque development in transgenic mice monitored by in vivo magnetic resonance microimaging. *J. Magn. Reson. Imaging* 24, 530–536
13 Leskovjan, A.C. *et al.* (2011) Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease. *Neuroimage* 55, 32–38
14 El Tayara, N.T. *et al.* (2007) Transverse relaxation time reflects brain amyloidosis in young APP/PS1 transgenic mice. *Magn. Reson. Med.* 58, 179–184
15 Waerzeggers, Y. *et al.* (2010) Mouse models in neurological disorders: applications of non-invasive imaging. *Biochim. Biophys. Acta* 1802, 819–839
16 Chamberlain, R. *et al.* (2011) Magnetic resonance imaging of amyloid plaques in transgenic mouse models of Alzheimer's disease. *Curr. Med. Imaging Rev.* 7, 3–7
17 Sigurdsson, E.M. *et al.* (2008) A non-toxic ligand for voxel-based MRI analysis of plaques in AD transgenic mice. *Neurobiol. Aging* 29, 836–847
18 Yang, J. *et al.* (2011) Detection of amyloid plaques targeted by USPIO-Abeta1-42 in Alzheimer's disease transgenic mice using magnetic resonance microimaging. *Neuroimage* 55, 1600–1609
19 Poduslo, J.F. *et al.* (2011) Targeting vascular amyloid in arterioles of Alzheimer disease transgenic mice with amyloid beta protein antibody-coated nanoparticles. *J. Neuropathol. Exp. Neurol.* 70, 653–661
20 Petiet, A. *et al.* (2011) Gadolinium-staining reveals amyloid plaques in the brain of Alzheimer's transgenic mice. *Neurobiol. Aging* (in press)
21 Higuchi, M. *et al.* (2005) 19F and 1H MRI detection of amyloid beta plaques in vivo. *Nat. Neurosci.* 8, 527–533
22 Teipel, S.J. *et al.* (2011) Automated detection of amyloid-beta-related cortical and subcortical signal changes in a transgenic model of Alzheimer's disease using high-field MRI. *J. Alzheimers Dis.* 23, 221–237
23 Benveniste, H. *et al.* (2007) Anatomical and functional phenotyping of mice models of Alzheimer's disease by MR microscopy. *Ann. N. Y. Acad. Sci.* 1097, 12–29
24 Badea, A. *et al.* (2010) Remote sites of structural atrophy predict later amyloid formation in a mouse model of Alzheimer's disease. *Neuroimage* 50, 416–427
25 Yang, D. *et al.* (2011) Volumetric MRI and MRS provide sensitive measures of Alzheimer's disease neuropathology in inducible Tau transgenic mice (rTg4510). *Neuroimage* 54, 2652–2658
26 Beckmann, N. *et al.* (2011) Noninvasive magnetic resonance imaging detection of cerebral amyloid angiopathy-related microvascular alterations using superparamagnetic iron oxide particles in APP transgenic mouse models of Alzheimer's disease: application to passive Abeta immunotherapy. *J. Neurosci.* 31, 1023–1031
27 Lifshitz, V. *et al.* (2011) Immunotherapy of cerebrovascular amyloidosis in a transgenic mouse model. *Neurobiol. Aging* (in press)
28 Luo, F. *et al.* (2010) Magnetic resonance imaging detection and time course of cerebral microhemorrhages during passive immunotherapy in living amyloid precursor protein transgenic mice. *J. Pharmacol. Exp. Ther.* 335, 580–588
29 Weidensteiner, C. *et al.* (2009) Cortical hypoperfusion in the B6.PS2APP mouse model for Alzheimer's disease: comprehensive phenotyping of vascular and tissular parameters by MRI. *Magn. Reson. Med.* 62, 35–45
30 Hooijmans, C.R. *et al.* (2007) Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched Typical Western Diet (TWD). *Neurobiol. Dis.* 28, 16–29
31 El Tayara, N.T. *et al.* (2010) Detection of vascular alterations by in vivo magnetic resonance angiography and histology in APP/PS1 mouse model of Alzheimer's disease. *MAGMA* 23, 53–64
32 Thal, D.R. *et al.* (2009) Capillary cerebral amyloid angiopathy is associated with vessel occlusion and cerebral blood flow disturbances. *Neurobiol. Aging* 30, 1936–1948
33 Kim, J. *et al.* (2011) Quantitative in vivo measurement of early axonal transport deficits in a triple transgenic mouse model of Alzheimer's disease using manganese-enhanced MRI. *Neuroimage* 56, 1286–1292
34 Smith, K.D. *et al.* (2011) R-flurbiprofen improves axonal transport in the Tg2576 mouse model of Alzheimer's disease as determined by MEMRI. *Magn. Reson. Med.* 65, 1423–1429
35 Choi, J.K. *et al.* (2007) Application of MRS to mouse models of neurodegenerative illness. *NMR Biomed.* 20, 216–237

36 Hess, A. *et al.* (2011) Blockade of TNF-alpha rapidly inhibits pain responses in the central nervous system. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3731–3736
37 Princz-Kranz, F.L. *et al.* (2010) Vascular response to acetazolamide decreases as a function of age in the arcA beta mouse model of cerebral amyloidosis. *Neurobiol. Dis.* 40, 284–292
38 Baltes, C. *et al.* (2010) Increased blood oxygen level-dependent (BOLD) sensitivity in the mouse somatosensory cortex during electrical forepaw stimulation using a cryogenic radiofrequency probe. *NMR Biomed.* 24, 439–446
39 Scheltens, P. and Rockwood, K. (2011) How golden is the gold standard of neuropathology in dementia? *Alzheimers Dement* 7, 486–489