Review Article

Assessment of multifunctional contrast agent probes in neuroimaging: Implications of nanopharmaceutical therapeutic interventions

Lina H. Machtoub

Innsbruck University Hospital, Clinic of Radiodiagnostic, A-6020 Innsbruck; Eduard Wallnoefer Zentrum, A-6060 Hall-Tirol, Austria

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Abstract

Recently, a clear association has been found between the progression of motor neurodegenerative disorders (MNDs) and carotid atherosclerosis. Significant vascular abnormalities with arterial hypertension were shown to be in patients with familial antecedents of MNDs. The main scope of this work is to explore the feasibility of recently developed integrated nano-based imaging modalities for the assessment of early diagnosis of the inflammatory processes associated with the neurological disorder syndromes, with the implication of recently developed nanopharmaceutical therapeutic interventions.

Key words: Central nervous system, nanoparticles, neurological disorders, oxides

INTRODUCTION

The combination of molecular imaging and neuroscience has recently made a great progress in the development of high sensitivity diagnostic targeted imaging probes for the non-invasive characterization of the molecular events associated with the inflammatory process in the vascular system. In this context, intensive studies have been working on the development of potential nanoparticles contrast agents for multimodal imaging. Based on their strong optical sensitivity in the near infrared (NIR) and visible region, nanoparticles can be used for contrast enhancement for many optical imaging techniques important in cellular imaging and diagnosis, including laser optoacoustic imaging system (LOIS), optical coherence tomography and confocal microscopy.

Address for correspondence:
Prof. Lina H. Machtoub,
Univ.klinik Radiodiagnostik, Eduard Wallnoefer Zentrum,
A-6060 Hall-Tirol, Austria.
E-mail: lina.machtoub@i-med.ac.at

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LOIS has been of interest as a feasible modality in clinical imaging since the photoacoustic effect was initially discovered in 1880.[1,2] By incorporating a targeting moiety into the contrast agents, LOIS can be used to obtain the molecular signatures of various diseases with high contrast and high spatial resolution. Although it is very difficult to image human brain directly with LOIS at present, the imaging of microvasculature in the established experimental animal model can provide more knowledge and information of neurological dysfunctions.

In this work, targeted contrast agents were used to investigate the inflammatory process associated with neurodegenerative disorders and vascular diseases using novel nano-based imaging modalities. The targeted contrast agents are becoming more valued due to their effective approach on the chronic disorders, enabling as well the visualization of the early changes of the diseases. Recently, several oxides nanoparticles based contrast agents have been investigated in order to characterize the acute and chronic diseases with the ongoing process of inflammation.[3-7] There have been several studies that employed nanoparticles of specific molecular, biological and some multifunctional features to characterize the inflammatory process in neurodegenerative disorders.[8,9] In our recent studies, it has been shown that the administration of ultra-small paramagnetic iron oxide nanoparticles (USPIOs) increased the contrast in monitoring USPIO-phagocytosed atherosclerotic plaques using surface-enhanced coherent anti-Stokes Raman scattering (SECARS) microscopy and magnetic resonance imaging (MRI).[6,9-13]

Based on recent clinical findings, a clear association has been reported between carotid atherosclerosis and the progression of motor neurodegenerative disorders (MNDs), which indicate that the central nervous system (CNS) inflammation may itself contribute to the development of vascular diseases. Further clinical case studies have shown high-levels of vascular cell adhesion molecule-1 (VCAM-1) in chronic active lesions as well as in blood and cerebrospinal fluid in patients diagnosed with multiple sclerosis (MS).[14,15]

To our knowledge, this work provides the first experimental report to investigate the feasibility of integrated LOIS with surface enhanced nano imaging microscopy, assisted by MR-nanoscale contrast agents. The focus of this study is to show the feasibility of multifunctional contrast agents for early diagnosis of neurological disorders syndromes using multimodal imaging based on the combination of optoacoustic imaging, surface enhanced microscopy and MRI with implications of promising nanopharmaceuticals therapeutic interventions.

MATERIALS AND METHODS

The experiments were performed on MND established experimental animal models of Sprague-Dawley (SD) rats and a wild type ([WT]-standard SD rats). Commercially available antibodies, labeled with ultra-small iron oxide nanoparticles (USPIO; MACS®, MiltenyiBiotec) cross linked to anti-CD4 antibodies (CLUSPIO) were intravenous (IV) injected into rats. The contrast agent designed for the atherosclerotic inflammatory model was USPIO-linked with antibody against VCAM-1 adhesion molecule (GuerbetResearch-France). After MRI, brains were isolated and post fixed in 4% paraformaldehyde for 48 hr at + 4°C. Animal experiments were surveyed by the committee for animal experimentation treated in accordance with the European Community Council Directive (Ref.Nr. 86/609/EEC) and the National Institutes of Health Guidelines.

Experiments using LOIS were performed utilizing pulsed optical illumination and ultra-wide band ultrasonic detection of resulting optoacoustic signal. The system employs a laser system including a Q-switched Nd:YAG laser, producing 532 nm laser pulses with a FWHM of 10 ns, a pulse repetition rate of 15 Hz. The system resolution as estimated on phantoms was 0.5 mm. LOIS can provide ultrasound-resolution images with intrinsic optical contrast in regions up to 5 cm deep, with *in vivo* detectable functional changes and disorders since these changes and disorders usually induce local optical contrast through changing blood volume and oxygenation.

The control experiments were first detected on phantoms using 1mL solution of USPIO injected into the gelatin. In case of the tissue experiments, the samples were attached to the gelatin holder. The *in vitro* experiments were conducted on brain tissues extracted from the established rat model i.v. injected with the contrast. Before laser irradiation, both the gelatin and the tissue samples were placed in a water chamber for signal detection with impedance match. Control experiments were conducted on brain tissues extracted from wild-type rat model and untreated samples.

The surface enhanced microscopy experiments were performed using NIR excitation of 1064-nm of mode-locked Nd: YVO4 (7 ps, 76 MHz) and Ti: sapphire laser (Coherent Mira HP, 3.5 W 3 ps, 700-1000 nm) combined with a tunable optical parametric oscillator that covers the frequency range (200-3600 cm^{-1}). The collinearly combined pump and stokes beams are sent into an inverted optical laser scanning microscope. The beams were scanned over the sample and focused by water immersion objective lens with 1.2 numerical apertures. The probe volume was reduced below the diffraction limit by tightly focusing the laser beams. The microscope is designed for the signal to be detected in both forward and epi-direction. The collected signal is filtered by band-pass filters and detected by a photomultiplier module. For surface enhanced stimulated vibrational scattering microscopy, the stoke pulse was intensity-modulated in time and the signal was obtained through the lock-in detection of the changes in intensity of the anti-Stoke. The images were recorded with a resolution of 5 sec total acquisition time for one frame of 512 × 512 pixels. The major experimental advantage of the application based on surface enhanced vibrational nanoimaging microscopy is the drastic signal enhanced in the vicinity of nanoparticles that can increase the detection sensitivity reaching the single-molecule level.

RESULTS

Three-dimensional brain images of CLUSPIO treated rat brain were obtained with the laser wavelength at 532 nm [Figure 1]. The 532 nm wavelength was selected because of the high absorption by USPIO contrast agent. The acoustic signals were evaluated for both control and USPIO-administered specimens. Experiments performed on untreated brain tissues extracted from the WT rat model have shown no significant indication of enhancement. To evaluate the response signal, experiments were performed on contrast treated phantoms, which have shown significant acoustic signal enhancement in amplitude of 5 times greater by the administration of the nanoparticles.

In this experiment, we tested the hypotheses that nanoparticles such as USPIO could produce photoacoustic waves in response to short-pulsed laser irradiation and demonstrated the feasibility of USPIO MR-based contrast agent using optoacoustic imaging modality as a screening test for investigation of degenerated brain disorders. In line with LOIS, experiments preformed on CLUSPIO contrast treated MND rat model, using surface

enhanced nanoimaging microscopy showed marked signal enhancement in the degenerated regions of the treated brain sections.[13-20] The results were further supported by MRI [Figure 1].[9,10]

In earlier findings, oxides based MR contrast agents were shown to have a potential to access inflammatory activity

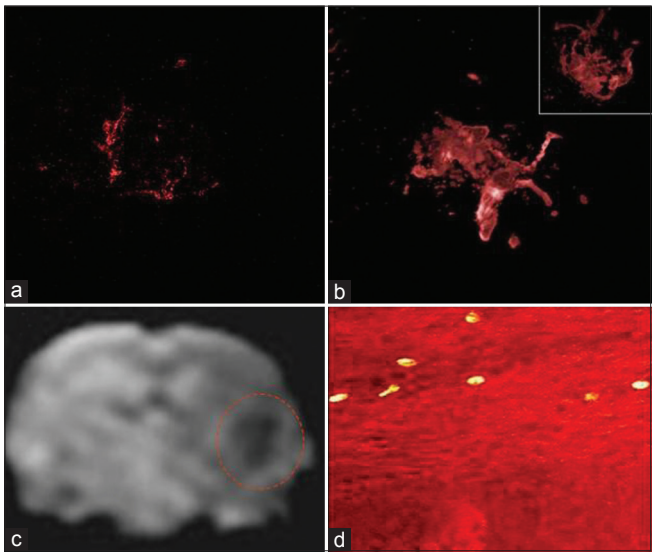

Figure 1: Laser optoacoustic imaging system 3D-brain images of ultra-small paramagnetic iron oxide nanoparticles (USPIO) treated rat brain obtained with the laser wavelength at 532 nm. (a) Before and (b) after administration of USPIO contrast agent (c) magnetic resonance image of (CLUSPIO) treated rat brain. T2* protocol reveals the hypointensities (note regions delimited by ellipsoids) allegedly caused by CLUSPIO seen with time to echo (TE) 25 ms. (d) Signal enhancement highlighting accumulation of iron oxide nanoparticles, bright structures, observed in tissues from CLUSPIO-treated ALS animals using surface enhanced nanoimaging based on stimulated vibrational microscopy

of cardiovascular diseases and CNS disorders.[12,13,21] The long blood circulating time and the progressive macrophage uptake of nanoparticles in inflammatory tissues are of the major importance in optical and MRI pathologic tissue characterization. In our recent studies, *ex vivo* SECARS experiments performed on USPIO-based contrast treated aortic sections showed markedly increased signal enhancements within atherosclerotic lesions predominantly in luminal regions of the plaque, highlighting intracellular iron uptakes in the endothelial and medial smooth muscle cells (SMCs). The results provided the first evidence that the signal loss observed by MRI may reflect iron deposits not only within the plaque and VCAM-1-expressing macrophages and endothelial cells, but also in the intimal and medial SMC, similarly displaying a strong upregulation of VCAM-1 in atherosclerosis [Figure 2].

In this context, using oxides based contrast agents (USPIO) in optoacoustic imaging can potentially increase the contrast and detection sensitivity within the visible range, which makes it a promising approach for clinical evaluation and early diagnosis of cerebrovascular abnormalities and neurological disorders.

DISCUSSION

In this work, the application of oxides contrast agents using multimodal imaging may assist in understanding the inflammatory process associated with neurodegenerative diseases and cerebrovascular disorders. Recently, clinical studies reported a clear association between the progression of MNDs and carotid atherosclerosis. In these studies, it has been shown that progression of a neurodegenerative disease in some patients could be correlated with the cardiovascular risk factors and carotid atherosclerosis. These cases were

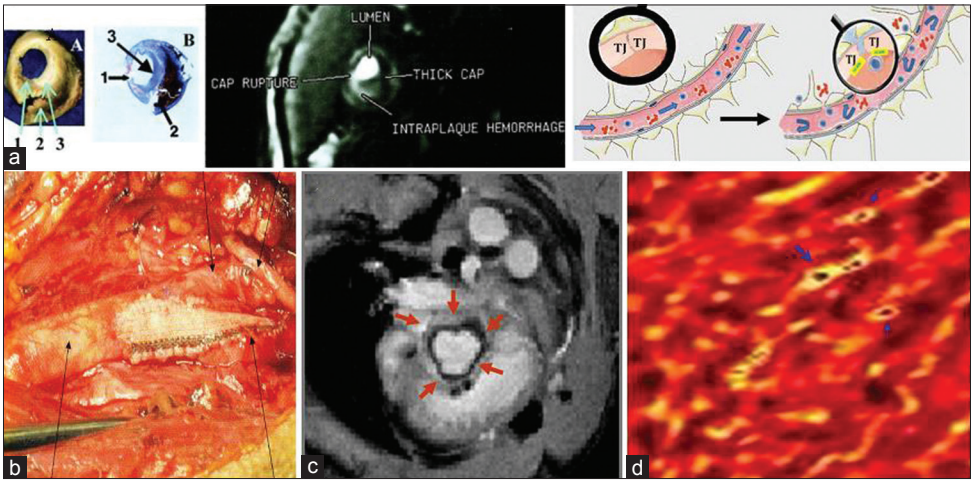

Figure 2: (a) Schematic diagram outlining the inflammatory process (b) Carotid endarterectomy, indicating major stenoses at the bifurcation of the common into the internal and external carotid arteries (arrow). (c) Ultra high field magnetic resonance imaging of the aortic roots of ApoE-/-mice, placed on a western-type diet for 26 weeks shows marked loss in signal intensity after administration of (USPIO-VCAM) contrast agent, illustrated by red arrows around the wall of the aortic root. (d) SECARS image shows intracellular iron uptakes in smooth muscle cells and endothelial cells

further supported in patients with familial antecedents of MND/Amyotrophic Lateral Sclerosis (ALS) diagnosed with significant vascular abnormalities and vascular risk factors, such as arterial hypertension, coronaropathy or hypercholesterolemia.[22]

These possible associated mechanisms were supported by previous studies on inflammatory models of CNS, which revealed that the loss of integrity of the blood-brain barrier is the fundamental event in the development of the CNS diseases. This was first illustrated by MRI, which detected a subtle blood-brain barrier leakage as an early finding in MS patients.[23] Though the exact mechanism of this blood-brain barrier leakage remains elusive, under inflammatory conditions, endothelial cells express adhesion molecules, primarily intercellular adhesion molecule 1(ICAM-1) that specifically bind their ligands and receptors on target leukocytes.[24] In a manner similar to the immune reactions in other tissues, activated lymphocytes travel through blood vessels of the brain and spinal cord, are captured by adhesion molecules expressed by endotheliocytes and finally transmigrate across the endothelial barrier. This, in turn, could lead to the increased permeability of the blood-brain barrier, allowing the adhesion of several subpopulations of leukocytes to the endothelium. Thus, expression of adhesion molecules by the brain and spinal cord endothelial cells is likely to be an essential step in the initiation of this inflammatory process and at least partially, responsible for the blood-brain barrier disruption (as, under normal conditions, cerebrovascular endothelium express very low levels of these proteins).[25,26]

From the data available, it is evident that combining different imaging modalities that are sensitive to different aspects of MND pathology using targeted contrast agents is a promising way to further increase our understanding of the inflammatory mechanisms accounting for the accumulation of irreversible disability in this condition.

Future Trends and Promising Therapeutic Interventions for Neurological Disorders

There have been intensive efforts to develop potential therapeutic options, either with substances that strengthen the tight-junctions barrier or with agents that reduce the expression of ICAM-1 and other adhesion molecules. In some reported cases, blood-brain barrier-targeted agents were shown to be promising for the treatment of neurological disorders. At the present time, it is likely that CNS gene therapy technology can dampen the symptoms of neural degeneration.[27]

Accordingly, extensive research studies are working on successful translation of a DNA vaccine to the clinical applications using effective intracellular delivery such as electroporation and gene gun mediated administration as the most effective physical methods for delivering DNA plasmids *in vivo*. Recent application studies using a delivery of DNA vaccine for Alzheimer's disease has been reported.[28] Despite promising results in animal models of MND and in several open label clinical investigations, the efficacy of gene therapy has yet to be confirmed in randomized clinical trials.

In ALS, at present, riluzole is the only approved drug that has been shown to have a modest effect on prolonging life expectancy in patients. A rapid increase of the N-acetyl aspartate/creatine ratio in the motor cortex in patients with ALS after only 1 day of riluzole treatment has been reported.[29] The current trends are working on the development of novel nanopharmaceuticals drugs with enhanced, focused effects, which can provide superior treatment for motor neuron diseases. Given that a nanoparticle may have the capacity to carry several molecules of a drug, an improvement in nanoparticles delivery to the CNS would contribute to more efficient delivery of pharmaceutical agents to the brain. In addition, the conjugation approach may also enhance the number of nanoparticles delivered selectively to toxic Ab aggregates, a crucial factor for the early diagnosis and therapy of neurodegenerative disorders.

Recently promising experiments are currently under way in investigating the potential therapeutic contribution of oxides based nanoparticles in the preservation of neuronal function and prevention of neurodegeneration. Oxides nanoparticles can play a crucial role in therapeutic interventions. It has been assumed that oxide based nanoparticle treatment may protect dopaminergic neurons from degeneration and death associated with chemical induction of Parkinson's-like disease, which can be promising for potential human treatment.[30] Current future studies are working on the possible pre-treatment with cerium oxide nanoparticles (CeONP) on pre-clinical models of Parkinson's disease. For this application, it is assumed that treatment of the model with CeONP appeared to reduce oxidative stress in the brain and increase striatal dopamine as well as the numbers of neurons in the substantia nigra. This suggests that oxides nanoparticles may be potent neuroprotective agents in Parkinson's disease.[30,31] In our recent studies, experiments using stimulated vibrational microscopy showed marked signal enhancement in the substantia nigra of treated brain sections of Parkinson rat model [Figure 3]. Thus, the administration of nanoparticles for the treatment of neurodegenerative disorders is becoming a promising therapeutic field. However, issues such as the transport mechanism across the blood-brain barrier, neurotoxic effects and the reduction of reticuloendothelial system RES interactions remain to be addressed.

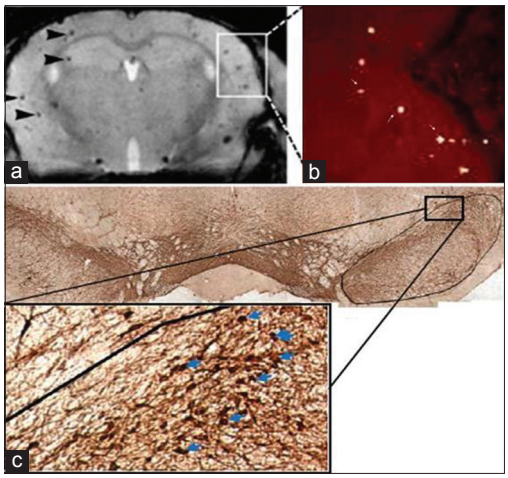

Figure 3: (a) Magnetic resonance image of Parkinson rat model. (b) Marked signal enhancement of the aggregated proteins, indicated by bright structures, observed in the brain of Parkinson rat model by using surface enhanced nanoimaging based on stimulated vibrational microscopy. (c) Corresponding light microscopic image of substantia nigra shows protein aggregation indicated by blue arrows

In summary, this study provides the first experimental report to investigate the feasibility of using photoacoustic imaging assisted by superparamagnetic nanoparticles to enhance imaging sensitivity in MND rat model. In this work, we showed that oxides based contrast agents facilitated significant contrast enhancement and potentially applicable for multimodal imaging in investigating neurological dysfunctions. A long term goal of these studies is to provide a unique perspective for understanding the molecular mechanism associated with neuorological disorders based on combined multimodal imaging of optical, optoacoustic and MRI. The unique properties of oxides, characterized by elevated optical absorption and magnetic susceptibility, make it a promising suitable diagnostic tool for monitoring the inflammation that may contribute substantially to pre-clinical and clinical evaluation of the cerebrovascular diseases and neurological disorders. Furthermore, the potential therapeutic contribution of oxides nanoparticles in the preservation of neuronal function and prevention of neurodegeneration can play a crucial role in therapeutic interventions and can be promising to limit neuroinflammation, cerebrovascular diseases and vascular dementia.

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