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Phase contrast radiography of Lewy bodies in Parkinson disease

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Parkinson's disease (PD), defined as a neurodegenerative disorder, is characterized by the loss of dopaminergic neurons and the presence of Lewy bodies in neurons. Morphological study of Lewy bodies is important to identify the causes and the processes of PD. Here, we investigate a possibility of phase contrast radiography using coherent synchrotron X-rays to explore the microscopic details of Lewy bodies in thick (~3 mm) midbrain tissues. Autopsied midbrain tissues of a PD patient were sliced in 3 mm thickness and then examined using synchrotron X-rays from the 7B2 beamline of the Pohang Light Source. Refraction-enhanced phase contrast radiography and microtomography were adopted to identify dark core and dim edge of Lewy bodies in neurons. The morphology of Lewy bodies was clearly revealed by the phase contrast radiography in very thick (3 mm) midbrain tissues without any staining treatment. Three-dimensional volume rendered microtomography of the autopsied midbrain tissues demonstrates striking evidence that several Lewy bodies are agglomerated by dim edges in a neuron. We suggest that the phase contrast radiography could be a useful tool to morphologically investigate the causes or the processes in PD.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of midbrain dopamine neurons and the presence of intracytoplasmic-ubiquitinated inclusions (Lewy bodies) in degenerating neurons (Spillantini et al., 1997; Feaby and Bender, 2000; Chung et al., 2001). PD is believed as a slowly progressive neurodegenerative disorder with no identifiable cause, followed by the loss of pigmented neurons and gliosis, most prominently in substantia nigra and locus ceruleus (Zgaljardic et al., 2004). Moreover, the morphology and the composition of Lewy bodies are important to identify the causes and the processes of PD in different portions of the nervous system, which has been one of the key issues of nerve cell degeneration in PD (Forno, 1987).

The underlying neuronal degeneration in PD is believed to occur slowly during the decades preceding the onset of symptoms. Up to 80% of dopaminergic neurons are lost before the early clinical symptoms of PD appear (Koller et al., 1991). However, diagnosis in living subjects is based entirely on the neurological examination, because there is no antemortem biologic marker for PD. A clinicopathological study showed that only 76% of patients with a clinical diagnosis of PD actually met the pathological criteria; the remaining 24% showed the evidences of other causes of parkinsonism (Hughes et al., 1993). Nowadays radiotracer imaging of the nigrostriatal dopaminergic system with PET- and SPECT-based ligands can additionally be used to diagnose PD. But current evidence dose not support the use of radiotracer imaging as diagnosis tool in clinical practice (Ravina et al., 2005).

Since the discovery of X-ray by Wilheim Conrad Röntgen in 1895, medical radiology has been far the most important application of X-rays. In the overwhelming majority of cases, the contrast in the radiological images is based on the different X-ray absorption by different parts of the specimen. Absorption is very limited for X-rays: this is the basis of the striking success of radiology but also of its limitations. Computed tomography (CT) and magnetic resonance imaging (MRI) can sometimes provide better image quality than conventional radiology (Lee et al., 2002). However, they cannot detect fine structures of several-micron size, useful for the early diagnosis of neurodegenerative disorders in the preclinical period.

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Recently, X-ray imaging technology based on phase contrast mechanisms has greatly advanced thanks to the use of unmonochromatic synchrotron hard X-rays for biological specimens (Hwu et al., 2004; Margaritondo et al., 2004). We expect that phase contrast radiography will be able to be appropriate for the microscopic details of Lewy bodies even in very thick midbrain tissues. The phase contrast radiography – a new conceptual approach to radiography – greatly increases the amount of information that can be obtained with radiographic techniques and is particularly well suited to the imaging of soft tissue and of very small features in biological samples (Hwu et al., 2004; Margaritondo et al., 2004). We examined the microstructure of midbrain in autopsied brain of a PD patient with phase contrast radiography. This study shows a possibility that phase contrast radiography can be used to explore the microscopic details of Lewy bodies even in thick midbrain tissues.

Subjects and methods

Patient history

In this study, we selected and focused a male adult who at the age of 53 years began to suffer from slowly progressing gait disturbance with left hand resting tremor, rigidity and bradykinesia. Even though the patient showed good response to levodopa, due to the typical progression of disease, he had to be hospitalized several times because of motor fluctuation, easy falling and dysphagia after the age of 61. The patient finally died at the age of 63 years after a 10-year illness due to pneumonia.

Pathology

A pallor and smeared appearance of reddish-brown discoloration in the substantia nigra was observed on the inspection of the cut space of autopsied midbrain (Fig. 1), as typical for PD. On microscopic examination, dopaminergic cell loss and Lewy bodies were clearly observed (Fig. 2).

Phase contrast X-ray

The autopsied midbrain tissues of a PD patient were sliced in 3 mm thickness for the phase contrast radiography. Fig. 3 schematically shows the typical imaging set-up. The experimental geometry and in particular the detector position were selected to emphasize the refraction-based mechanism (Hwu et al., 2004; Margaritondo et al., 2004). The midbrain tissue was typically placed 200–400 μm from the detector to achieve the best contrast. The detector system consisted of a thin (150 μm) $CdWO_4$ cleaved single crystal scintillator and of a CCD camera. A microscope objective lens magnified the image displayed on the scintillator before it was captured by the CCD.

The fields of view of images were 500 × 375 and 500 × 500 μm^2 for microradiology and microtomography experiments, respectively. The exposure and the image acquisition times were 100 ms. In order to look for the Lewy bodies from radiography experiments, we first scanned the sample by 5 × 5 mm^2 and then patched the images automatically. From the patched image, we were able to look for the Lewy bodies quickly. The microtomography experiments were conducted by taking images from the sample by rotation of 180° with every 0.18° increment of rotation. The reconstruction of the image set was carried out by computed reconstruction using four parallel computers equipped with a reconstruction algorithm. It took 4 h for the whole procedure of microtomography. The Lewy bodies were able to be searched within several minutes by 3D volume rendering.

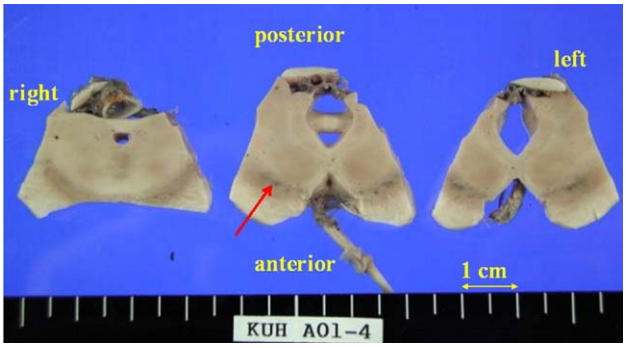

Fig. 1. A pallor and smeared appearance of reddish-brown discoloration in the substantia nigra (arrow) were observed on the inspection of the cut space of autopsied midbrain.

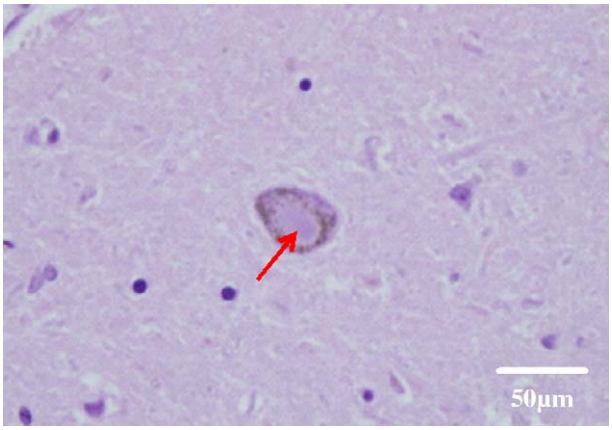

Fig. 2. On microscopic examination, dopaminergic cell loss and Lewy bodies (arrow) were clearly observed (H&E stain).

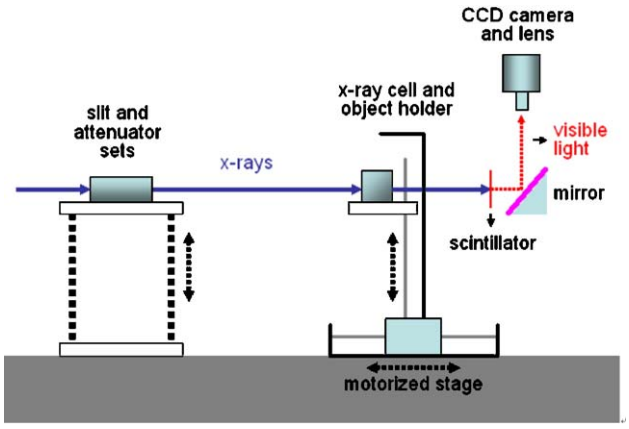

Fig. 3. The schematic diagram of the end station for synchrotron X-ray imaging. The key feature of the 7B2 beamline of the Pohang Light Source (PLS) is the use of white or unmonochromatic X-rays to achieve very high lateral and time resolution.

Phase contrast radiography was performed using the highly coherent synchrotron hard X-rays at the ICPCIR (International Consortium of Phase Contrast Imaging and Radiology) (7B2) beamline of the Pohang Light Source in Korea (Baik et al., 2004). The hard X-rays used here are ranged between 10 and 60 keV. As to tissue radiation dosage, phase contrast method is very advantageous compared with conventional absorption one. The main reason is because phase contrast enhancement is much more sensitive than absorption one for hard X-rays as already reported (Margaritondo et al., 2004). In addition, unnecessary X-ray irradiation is cut off by using a high-speed (1 ms) X-ray shutter and silicon attenuators.

Results

The central finding in this study is that the presence of Lewy bodies in remaining neurons is clearly observed on the phase contrast radiography and microtomography images without any staining treatment, as demonstrated in Fig. 4. The red arrows indicate dark cores of Lewy bodies in Fig. 4(a). Interestingly the presence of three Lewy bodies in remaining neurons was clearly observed in very thick (3 mm) midbrain tissues by the non-destructive, three-dimensional phase contrast X-ray imaging techniques. The white boundary in Fig. 4(a), which was formed by the phase contrast, indicates the neuronal cell body. Even though the contrast is weak, dim edges can be seen around the dark cores. Furthermore, from the three-dimensional volume rendered microtomography image of the autopsied midbrain tissues in Fig. 4(b), we obtained striking evidence that dark cores of Lewy bodies are agglomerated by dim edges in a neuron. It is noteworthy that three-dimensional distributions of the dark cores of the Lewy bodies are clearly seen as well. One or two Lewy bodies were observed in one field of view sized with 600 × 1100 μm.

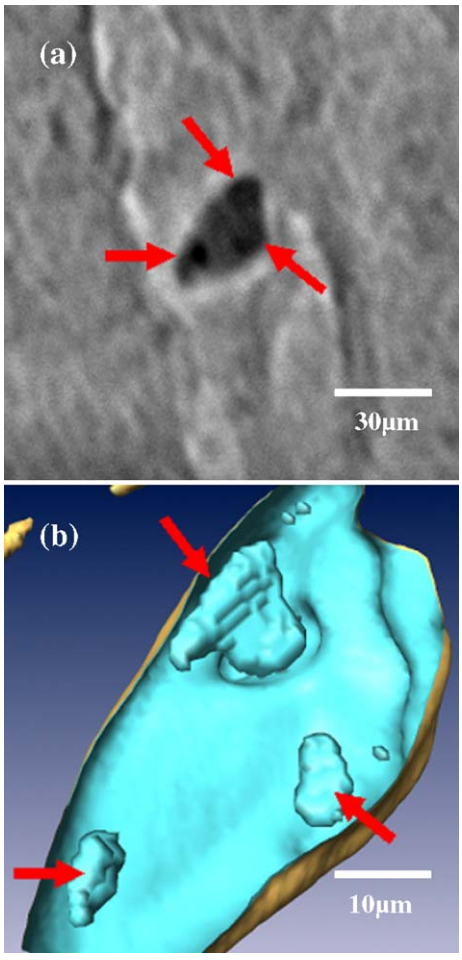

Fig. 4. The presence of Lewy bodies in remaining neurons is clearly observed on phase contrast X-ray microscopy images: (a) Lewy bodies appear to be inclusions like dark core within dim edge in size (5–30 μm). The red arrows point out to dark cores, which are considered as the Lewy bodies. (b) The three-dimensional volume-rendered microtomography image shows the three Lewy bodies in a neuron.

Discussion

Clinicopathological studies affirm diagnostic problems in assessing patients with parkinsonism. In 1992, Hughes at al. found that only 76 of 100 patients clinically diagnosed as having PD exhibited nigral Lewy bodies at autopsy; neuropathological diagnoses on the remaining 24 cases included progressive supranuclear palsy, multiple system atrophy and Alzheimer's disease (Hughes et al., 1993). The identification of the morphology and composition of Lewy bodies is the heart problem to reveal the causes and processes of PD in different portions of the nervous system (Forno, 1987). With the advent of magnetic resonance imaging (MRI) and functional imaging including radiotracer imaging, the role of neuroimaging in the diagnosis of Parkinsonian disorders is becoming more important (Eidelberg and Edwards, 2000; Fukuyama, 2004). MRI findings in Parkinsonian disorders can be helpful in exclusion of structural lesions such as basal ganglia tumor, hemorrhages, small vessel disease, and calcification (Seibyl et al., 2004). Functional imaging provides a sensitive means of detecting subclinical disease with biomarker. However, both MRI and functional imaging cannot be a confirmative diagnostic tool in PD and other related movement disorders (Ravina et al., 2005).

For the past 100 years, X-ray radiography has used absorption as the sole means of contrast formation and ray optics or computed tomography as the basis for image formation (Beckmann et al., 1997). A new conceptual approach to X-ray radiography is based on phase contrast and requires wave optics for proper treatment. This new approach greatly increases the amount of information that can be obtained with radiographic techniques and is particularly well suited to the imaging of soft tissue and of very small features in biologic samples (Meuli et al., 2004). Phase contrast radiography offers a number of improvements over conventional radiography in a clinical setting (Eidelberg and Edwards, 2000; Takeda et al., 1998a,b). These improvements, including use of harder X-ray and increased contrast result in improved visualization of anatomic detail, reduced absorbed dose to the patient, inherent image magnification and high spatial resolution and relative ease of implementation. More technologically advanced detectors are currently being developed and commercialized, which will help fully realize the considerable potential of phase-contrast radiography. Phase contrast X-ray provides a novel solution with the potential to create the biggest change in medical X-ray imaging since the invention of computed tomography (Gao et al., 1998).

Phase contrast radiography is based on the phase contrast enhancement mechanisms, which is expected to be useful to

identify the morphology (particularly, core and edge) of Lewy bodies. The phase contrast mechanisms are based on the observation of the interference pattern between diffracted and undiffracted waves produced by spatial variations of the real part of the complex refractive index (Hwu et al., 2004; Margaritondo et al., 2004). Highly coherent synchrotron sources enhance the phase contrast mechanisms. Particularly, the hard X-rays at 7B2 beamline in the PLS, with a large penetration depth, provide non-destructive imaging of thick opaque samples with a spatial resolution of submicron level (Hwu et al., 2004; Margaritondo et al., 2004). The phase contrast radiography is very sensitive to density and refraction gradients in the object. Thus, it can easily image weakly absorbing materials such as biological specimens.

In particular, Hwu et al. reported that the phase contrast radiography was appropriate for the imaging of biological specimens (aloe lead) as thick as 5 mm with showing cellular level details (Hwu et al., 2004; Margaritondo et al., 2004). This advantage of phase contrast radiography for assessability of very thick specimens would be very useful to investigate the morphological evolution of Lewy bodies in neurons. The conventional techniques such as TEM or SEM never detect such thick specimens.

Phase contrast radiography has many advantages over conventional radiography. The first advantage is very high contrast and time resolution with lower dose of X-ray. The second is good spatial resolution with better than 1 μm. The third is real time resolution radiography.

Phase contrast radiography also has problems to solve. The phase contrast factor was not included in the reconstruction algorithm used in this work. In spite of that, the phase contrast indeed plays an important role in the microtomography mostly because the phase contrast induces edge enhancement, which is able to recognize detailed structure in tissue rather easily. Currently, the phase retrieval study is under way to retrieve the phase and the projected attenuation from the acquired data in order to achieve the quantitative data.

Finally we discuss the feasibility of phase contrast radiography and microtomography in clinical application to live patents. For this purpose, significant improvements in phase contrast radiography are required in several aspects. The first is the development of very high sensitive detector with high time resolution (for instance, 1 μs) to solve out the problems raised by the dynamic movement of organs in live patients. High time resolution is again associated with the significant reduction of the total radiation dose (Margaritondo et al., 2004). The next is the development of compact point X-ray sources with high collimation, which enables to carry out microtomography in 1-μm resolution just by applying them to conventional computed tomography medical systems.

Conclusion

The major findings in the present study are that the phase contrast radiography clearly visualizes Lewy bodies consisting of dark core and dim edge even in thick tissue (3-mm thickness) without any staining treatment. In addition, the phase contrast radiography enables us to investigate three-dimensional distributions of the dark cores of the Lewy bodies in the thick specimen with microtomography techniques. The limitations of the conventional techniques are above all the requirements of (i) very thin slices as specimen and (ii) complicated and time-consuming staining procedures. Consequently, this study presents a possibility that phase contrast radiography can be used to explore the microscopic details of Lewy bodies even in thick midbrain tissues. To our knowledge, this report is the first success for non-destructive, three-dimensional images of the agglomerated Lewy bodies in a neuron cell using phase contrast radiography.

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