Minireview

Nanoscale biogenic iron oxides and neurodegenerative disease

Jon Dobson*

Centre for Science and Technology in Medicine, Department of Biomedical Engineering and Medical Physics, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent ST4 7QB, UK

Received 28 February 2001; revised 28 March 2001; accepted 2 April 2001

First published online 12 April 2001

Edited by Jesus Avila

Abstract One of the characteristics of many neurodegenerative diseases is the disruption of normal iron homeostasis in the brain. Recent experimental work indicates that nanoscale magnetic biominerals (primarily magnetite and maghemite) may be associated with senile plaques and tau filaments found in brain tissue affected by these diseases. These findings have important implications for our understanding of the role of iron in neurodegenerative disease as well as profound implications for their causes. In addition, the presence of biogenic magnetite in affected tissue should also provide improved mechanisms for early detection through the modification of MRI pulse sequences.

Key words: Amyloid; Alzheimer's disease; Neurodegenerative disease; Parkinson's disease; Magnetite; Ferritin

1. Introduction and background

Though iron plays an important role in virtually all living organisms – primarily through electron transport due to its ability to change valence – it has a rather limited bioavailability and, in some situations, it also can be toxic to cells. For this reason, it is necessary for organisms to sequester iron in a non-toxic form. In the human body (including the brain), as well as in most organisms, iron is stored primarily in the core of the iron storage protein ferritin. The ferritin protein is a hollow spheroid shell 12 nm in diameter made up of 24 subunits. The central void in the shell is 8 nm in diameter and is normally occupied by the iron biomineral ferrihydrite – a hydrated iron oxide ($5Fe_2O_3{\cdot}9H_2O$) which generally contains only Fe(III). It is in this form that most of the iron in the body is stored.

In humans disruption of normal iron metabolism in the brain is a characteristic of several neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and progressive supranuclear palsy (PSP). For example, excess iron accumulation is known to occur in AD patients – particularly in AD plaques (e.g. [1]) and total iron levels are elevated in the hippocampus, amygdala, nucleus basalis of Meynert and the cerebral cortex [2,3]. These elevated iron levels in neurodegenerative tissue, however, do not correlate with elevated levels of ferritin or the extracellular iron transport protein transferrin [3]. In fact, in several regions of the brains of AD and PD patients, a reduction in transferrin, indicating reduced mobility and sequestration of iron, has been reported [3,4].

Recent studies have shown that various forms of iron may play a significant role in the biochemical processes which lead to the progression of these diseases. This is primarily thought to be a result of oxidative stress – the generation of free radicals via the Fenton reaction [5–8]. However, other results also suggest that ferritin may act to modulate the formation of tau filaments in PSP [9] (though there are no reliable indications of abnormal ferritin levels in neurodegenerative tissue) and that iron may promote aggregation of betaA4 [10].

Though the association of anomalous concentrations of iron with neurodegenerative tissue is well documented, particularly in AD, methods for assaying iron in diseased tissue generally are ion specific, have poor spatial resolution and provide little reliable quantitative information [11]. Recently, some progress has been made in high-resolution iron analysis of AD tissue by Smith and others [7,8]. They have shown, using modified iron staining techniques, that redox-active iron is closely associated with AD plaques and neurofibrillary tangles. This work also has demonstrated that lesion-associated iron is distinct from iron sequestered in ferritin and has provided indirect evidence of the presence of Fe(II) in AD tissue.

Though this technique offers improved resolution, it is still not possible to identify the structural form of the iron or to map Fe(II) distribution. As such, iron anomalies associated with neurodegenerative disease are not well characterized and the structural/molecular form of the excess iron in AD plaques and neurodegenerative tissue in general, is not known.

Research in this field is, however, beginning to shed light on the role of iron. For example, work published last year has demonstrated the further strengthened connection between high levels of iron in the basal ganglia and oxidative stress in Parkinson's patients [12]. A disruption of iron metabolism and increased iron in this same region of the brain also has been implicated in AD and Huntington's disease [13]; and iron accumulation has been associated with microgliosis and correlated with increased damage to the CA1 region of the hippocampus via iron–zinc interactions in models of neurodegenerative diseases [14].

It should be noted, however, that some results have shown that the presence of oxidized nucleosides in neurons does not appear to be related to senile plaque material or neurofibrillary tangles in AD [15], though there are indications that iron

*Fax: (44)-1782-717 079. E-mail: jdobson@keele.ac.uk

0014-5793/01/$20.00
PII: S0014-5793(01)02386-9

is a significant source of oxidative damage in AD [8]. In some cases, free radical damage may even be reduced by Aβ deposition due to the inhibitory role of Aβ-related Zn^{2+} in H_2O_2-mediated toxicity [16,17].

Though ferritin is the primary mechanism for iron storage in the brain, over the past decade experimental work has demonstrated the presence of another form of iron in human brain tissue – biogenic magnetite (Fe_3O_4). Some of the potential consequences of the presence of magnetite in neurodegenerative disease tissue will be examined here.

2. Biogenic magnetite in the human brain

Biogenic magnetite, along with maghemite (γFe_2O_3, an oxidation product of magnetite with very similar magnetic properties which is likely produced by oxidation of magnetite during ex vivo tissue handling), were first discovered in human brain tissue in 1992 by a group at the California Institute of Technology led by Joseph Kirschvink [18]. This work concentrated on studies of human brain tissue samples taken from cadavers and proved somewhat controversial.

In order to examine the possibilities of contamination and post mortem changes in brain chemistry, our group undertook a series of studies on tissue removed from the human hippocampus [19]. As these examinations were performed on tissue resected during amygdalohippocampectomies (a surgical procedure in which the damaged hippocampus of focal epilepsy patients is removed) as well as cadaver tissue, post mortem artefacts could be controlled. The results demonstrate clearly that biogenic magnetite is present in human brain tissue and confirm Kirschvink's earlier results [20–22].

Magnetite is a ferrimagnetic iron oxide with alternating lattices of Fe(II) and Fe(III) which are antiferromagnetically coupled. This alternation of lattices and their corresponding differences in the number of unpaired electron spins give magnetite its strong magnetization. Since the discovery of magnetite in the human brain, particles of this material have been extracted, imaged and characterized both magnetically and morphologically through transmission electron microscopy (TEM) (see Fig. 1) and superconducting quantum interference device (SQUID) magnetometry [18–24].

The particles are generally smaller than 200 nm and, in most cases, are on the order of a few tens of nanometers. While some particles exhibit dissolution edges, others preserve pristine crystal faces and all particles examined thus far are chemically pure (this is common in biogenic magnetite). Morphologically, the particles are similar to those observed in magnetotactic bacteria (e.g. [25]) and magnetic analysis of bulk tissue samples indicates that the particles are likely present in magnetically interacting clusters.

Unfortunately, up to now these particles have only been observed in tissue extracts and we are currently developing new techniques for imaging the particles in tissue slices and mapping their distribution to tissue structures.

3. Ferritin as a magnetite precursor?

The ferrihydrite core of ferritin is capable of safely storing up to 4500 iron atoms which, under normal circumstances, are rendered unreactive with other molecules within the cell due to the protein cage barrier [26]. Iron is moved into and out of the ferritin shell through 3-fold and 4-fold channels between the protein's subunits. Sequestration is thought to be primarily an oxidative process as highly toxic Fe(II) is taken into the protein and oxidized to be stored as less toxic Fe(III) in the form of ferrihydrite [27]. As the amount of iron stored in the ferritin core is somewhat variable, the increases in iron observed during histological examination of neurodegenerative tissue may be due to increases in the number of iron atoms stored in the core of the protein.

While an increase in the number of iron atoms in the ferritin core may account for some of the excess iron observed in histological examinations, another form of iron appears to be present which could have significant consequences for neurodegenerative disease progression and early detection. If the ferritin core becomes overloaded or there is a breakdown in the protein's function, a mechanism for Fe(II) oxidation is lost. This process could lead to the formation of biogenic magnetite, which is more strongly magnetic (ferrimagnetic) than ferrihydrite (a superparamagnetic antiferromagnet at body temperature) and contains alternating lattices of toxic Fe(II) and less toxic Fe(III).

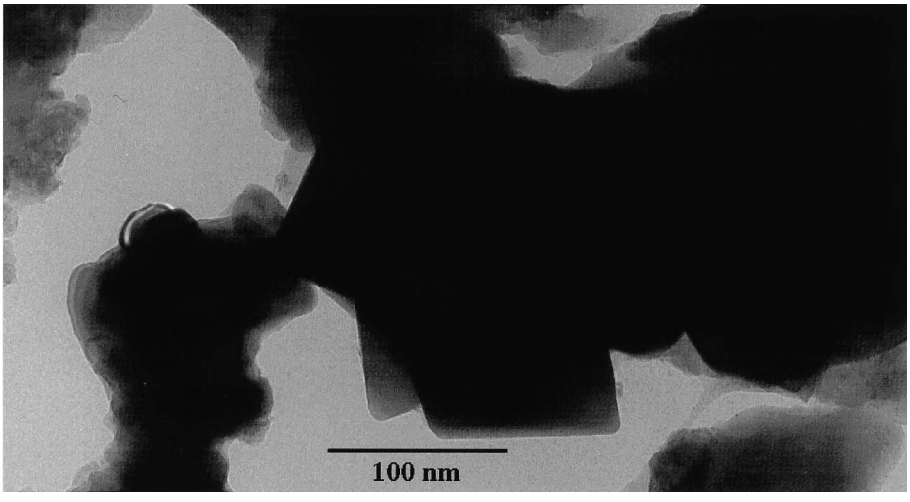

Fig. 1. TEM micrograph of biogenic magnetite extracted from the human hippocampus (figure after Schultheiss-Grassi et al., 1999).

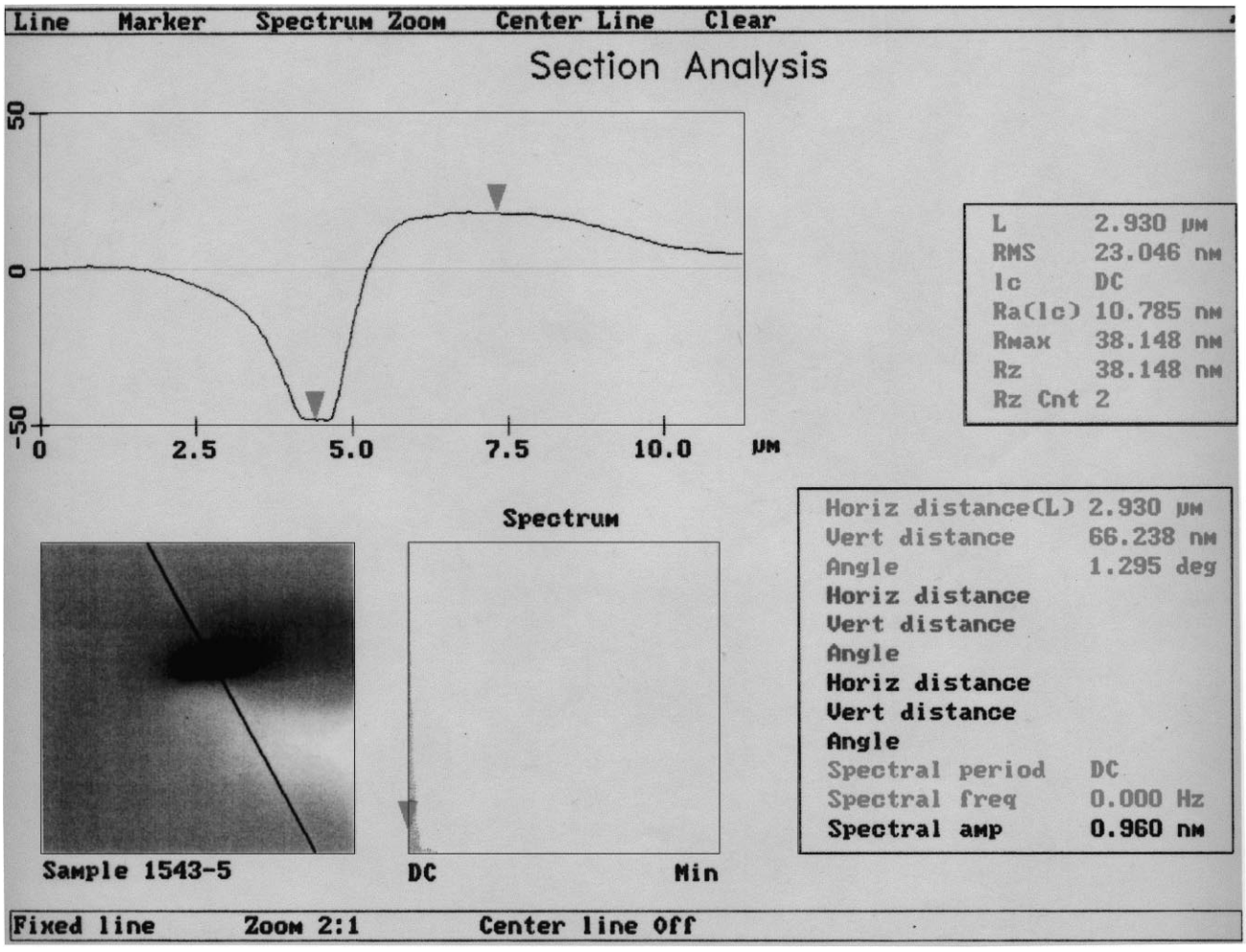

Fig. 2. Magnetic force microscope scan of plaque material from the human hippocampus showing a dipole-like magnetic response. (Figure after Dunn et al., 1995, [20].)

4. Potential consequences of biogenic magnetite in neurodegenerative tissue

Preliminary experimental studies by our group and the group of Dr. Carmen Quintana at the Instituto de Microelectronica de Madrid, suggest that this biogenic magnetite may be present in AD plaques, senile plaques and aberrant tau filaments extracted from PSP tissue [28]. In addition, magnetic analyses of relatively large (several grams) samples of AD tissue by Kirschvink's group indicate the presence of magnetite and/or maghemite, though the plaques were not analyzed separately [18].

In 1995 we used magnetic force microscopy (MFM) to examine a sample of hippocampal tissue which contained plaque material [20]. This opaque material exhibited a dipole-like response, consistent with the presence of magnetic material such as magnetite and/or maghemite (both have similar magnetic properties) (Fig. 2). Ferritin, which is superparamagnetic (i.e. does not behave like a 'magnet' at body temperature), would not produce such a response.

In late 1999 Dr. Quintana's group used high-resolution transmission electron microscopy and electron energy loss spectroscopy to examine ferritin in paired helical filaments from AD tissue and ferritin bound to aberrant tau filaments in neurodegenerative progressive supranuclear palsy. The results give a preliminary indication of the presence of a cubic iron oxide within the ferritin protein cage with spectra similar to synthetic magnetite/maghemite standards [28]. As discussed previously, it is possible that ferritin may act as a precursor for the formation of biogenic magnetite in humans, perhaps through excess loading of iron in the core and the breakdown of normal protein function. This is supported by the evidence for magnetite inside the ferritin protein cage.

If the presence of biogenic magnetite in AD plaques and neurodegenerative tissue is confirmed, it could have important consequences for our understanding of neurodegenerative disease progression – possibly even initiation – and could allow early detection and diagnosis.

Biogenic magnetite may play a role in the progression and initiation of neurodegenerative disease through free radical production leading to tissue damage at the site of magnetite accumulation. High levels of free iron in AD-affected brain tissue already have been noted as a possible cause of neuron degeneration through free radical processes via the Fenton reaction [5,29]. Magnetite and maghemite nanoparticles, however, also have been shown to have a substantial effect on free radical generation [30,31]. Recent experimental results also have demonstrated that iron–oxygen complexes may be a more effective catalyst for free radical damage in brain tissue than the Fenton reaction [32].

These effects are achieved through strong, local magnetic fields generated by biogenic magnetite particles which stabilize triplet states during biochemical reactions taking place nearby. This leads to the production of membrane-damaging free radicals and changes in reaction yields (e.g. [33]). Even relatively weak magnetic fields can have a strong influence on reaction yields [34]. In addition, Fe(II) in magnetite can be readily oxidized (forming maghemite) and this process, together with local magnetic field effects, may influence β-amyloid production and aggregation. This is particularly relevant considering studies showing that iron promotes aggregation of β-amyloid peptides in vitro and that β-amyloid potentiates free radical formation by stabilizing ferrous iron [10,35].

5. Biogenic magnetite and early detection of neurodegenerative disease

In addition to potential implications for disease progression, there are also possible benefits to the presence of magnetite in neurodegenerative tissue. Early detection is one of the primary goals of neurodegenerative research efforts and one of the methods being considered at present is the use of MRI. Usually, in more advanced patients, MR images exhibit regions of *hyper*intensity due to atrophy in the affected area of the brain (e.g. [36]). While this method can be successful in identifying neurodegenerative diseases such as Alzheimer's disease, by the time it is observed the disease has already progressed significantly from its early stages.

Several authors, however, have reported the appearance of *hypo*intensity artefacts in T2-weighted MR images of different areas of the human brain. Such artefacts have been observed in MRI studies of patients suffering from age-related neurodegenerative diseases, particularly Parkinson's disease and AD [37–39]. High-field (3 Tesla) MRI analysis of patients with various stages of Parkinsonian symptoms has even revealed a strong correlation between the severity of the symptoms and proxy measures of iron concentration based on T_2* values [39]. However, Chen and others have demonstrated that hypointensity artefacts do not correlate with excess levels of ferritin in the brain [40].

It is possible that these hypointensity artefacts may be explained by the effects of strong local magnetic fields generated by clusters of magnetite/maghemite in AD plaques and neurodegenerative tissue [18]. The mechanisms by which such artefacts could occur are well understood and biocompatible synthetic magnetite nanoparticles have been used as contrast agents in MRI (e.g. [41,42]). In addition, major advances in MRI technology already have been made which enable the quantitative mapping of iron by MRI methods [43]. If biogenic magnetite is present in AD plaques and neurodegenerative tissue, these methods could be adapted to look for regions of magnetite accumulation in subjects who may be predisposed to these diseases. This could lead to techniques for detection of neurodegenerative disease at a much earlier stage than is currently possible rather than imaging atrophied tissue already in the advanced stages of the disease.

6. Discussion

Histological examinations have shown consistently that excess iron is associated with pathological tissue in neurodegenerative diseases. As these increases in iron are not necessarily correlated to increases in ferritin, another form of iron is likely present. Experimental results thus far indicate that the accumulation of excess iron in this tissue has likely resulted in the formation of biogenic magnetite and or maghemite.

The presence of biogenic magnetite in AD plaques could have far-reaching consequences for our understanding of the disease. Though this material has not been observed – either directly or indirectly – specifically in AD plaques, there is ample reason to believe that it is probably there:

- Magnetite and maghemite are certainly present in other regions of the brain and the concentrations, packing geometries and grain sizes vary considerably.
- Iron levels in AD plaques cannot be adequately explained by increases in ferritin or transferrin.
- Preliminary experimental evidence indicates that some brain plaque material is magnetic and that some AD ferritin may contain magnetite.
- Magnetic analyses of relatively large AD tissue samples indicate the presence of magnetite and/or maghemite.

If magnetite is present in neurodegenerative tissue, this should lead to a better understanding of the role of this material in these diseases and may provide a mechanism for early detection and diagnosis of diseases that are notoriously difficult to diagnose at an early stage.

Acknowledgements: This research is partially funded by the University of Florida Opportunity Fund and the North Staffordshire Medical Institute. The author would like to thank Dr. Tim St. Pierre, Dr. Chris Exley and Prof. Chris Batich for helpful discussions and two anonymous reviewers for comments and suggestions which improved the manuscript.

References

[1] Lovell, M.A., Robertson, J.D., Teesdale, W.J., Campbell, J.L. and Markesbery, W.R. (1998) J. Neurol. Sci. 158, 470–520.
[2] Beard, J.L., Connor, J.R. and Jones, B.C. (1993) Nutr. Rev. 51, 157–170.
[3] Fisher, P., Gotz, M.E., Danielczyk, W., Gsell, W. and Riederer, P. (1997) Life Sci. 60, 2273–2278.
[4] Loeffler, D.A., Connor, J.R., Juneau, P.L., Snyder, B.S., Kanaley, L., Demaggio, A.J., Nguyen, H., Brickman, C.M. and Lewitt, P.A. (1995) J. Neurochem. 65, 710–716.
[5] Markesbery, W.R. (1997) Free Radic. Biol. Med. 23, 134–147.
[6] Connor, J.R. and Menzies, S.L. (1995) J. Neurol. Sci. 134, 33–44.
[7] Smith, M.A., Harris, P.L.R., Sayre, L.M. and Perry, G. (1997) Proc. Natl. Acad. Sci. USA 94, 9866–9868.
[8] Sayre, L.M., Perry, G., Harris, P.L.R., Liu, Y.H., Schubert, K.A. and Smith, M.A. (2000) J. Neurochem. 74, 270–279.
[9] Perez, M., Valpuesta, J.M., Montejo de Garcini, E., Quintana, C., Arrasate, M., Lopez-Carrascosa, J.L., Rabano, A., Garcia de Yebenes, J. and Avila, J. (1998) Pm. J. Pathol. 152, 1531–1539.
[10] Mantyh, P.W., Ghilardi, J.R., Rogers, S., Demaster, E., Allen, C.J., Stimson, E.F. and Maggio, J.E. (1993) J. Neurochem. 61, 1171–1174.
[11] Perl, P.P. and Good, P.F. (1992) Ann. Neurol. 32, S76–S81.
[12] Floor, E. (2000) Cell. Mol. Biol. 46, 709–720.
[13] Bartzokis, G. and Tishler, T.A. (2000) Cell. Mol. Biol. 46, 821–834.
[14] Shoham, S. and Youdim, M.B.H. (2000) Cell. Mol. Biol. 46, 743–750.
[15] Nunomura, A., Perry, G., Pappolla, M.A., Wade, R., Hirai, K., Chiba, S. and Smith, M.A. (1999) J. Neurosci. 19, 1959–1964.
[16] Cuajungco, M.P., Goldstein, L.E., Nunomura, A., Smith, M.A., Lim, J.T., Atwood, C.S., Huang, X., Farrag, Y.W., Perry, G. and Bush, A.I. (2000) J. Biol. Chem. 275, 19439–19442.
[17] Nunomura, A., Perry, G., Pappolla, M.A., Friedland, R.P., Hir-

ai, K., Chiba, S. and Smith, M.A. (2000) J. Neuropathol. Exp. Neurol. 59, 1011–1017.
[18] Kirschvink, J.L., Kobayashi-Kirschvink, A. and Woodford, B.J. (1992) Proc. Natl. Acad. Sci. USA 89, 7683–7687.
[19] Dobson, J. and Grassi, P. (1996) Brain Res. Bull. 39, 255–259.
[20] Dunn, J.R., Fuller, M., Zoeger, J., Dobson, J., Heller, F., Caine, E. and Moskowitz, B. (1995) Brain Res. Bull. 36, 149–153.
[21] Schultheiss-Grassi, P.P., Wessiken, R. and Dobson, J. (1999) Biochim. Biophys. Acta 1426, 212–216.
[22] Schultheiss-Grassi, P.P. and Dobson, J. (1999) BioMetals 12, 67–72.
[23] Kirschvink, J.L., Kobayashi-Kirschvink, A., Diaz-Ricci, J.C. and Kirschvink, S.J. (1992) Bioelectromagnetics 1 (Suppl.), 101–113.
[24] Kobayashi, A. and J.L. Kirschvink (1995) in: Electromagnetic Fields: Biological Interactions and Mechanisms (M. Blank, Ed.), Advances in Chemistry Series No. 250, pp. 367–394, American Chemical Society, Washington, DC.
[25] Dunin-Borkowski, R.E., McCartney, M.R., Frankel, R.B., Bazylinski, D.A., Posfai, M. and Buseck, P.R. (1998) Science 282, 1868–1870.
[26] Allen, P.D., St. Pierre, T.G., Chua-anusorn, W., Ström, V. and Rao, K.V. (2000) Biochim. Biophys. Acta 1500, 186–196.
[27] Harrison, P.M. and Arosio, P. (1996) Biochim. Biophys. Acta 1275, 161–203.
[28] Quintana, C., Lancin, M., Marhic, C., Pérez, M., Avila, J. and Carrascosa, J.L. (2000) Cell. Mol. Biol. 46, 807–820.
[29] Cornett, C.R., Markesbery, W.R. and Ehmann, W.D. (1998) Neurotoxicology 19, 339–345.
[30] Chignell, C.F. and Sik, R.H. (1998) Photochem. Photobiol. 65, 598–606.
[31] Scaiano, J.C., Monahan, S. and Renaud, J. (1997) Photochem. Photobiol. 65, 759–762.
[32] Schafer, F.Q., Qian, S.Y. and Buettner, G.R. (2000) Cell. Mol. Biol. 46, 657–662.
[33] Timmel, C.R., Till, U., Brocklehurst, B., McLauchlan, K.A. and Hore, P.J. (1998) Mol. Phys. 95, 71–89.
[34] Brocklehurst, B. and McLauchlan, K.A. (1996) Int. J. Radiat. Biol. 69, 3–24.
[35] Yang, E.Y., Guo-Ross, S.X. and Bondy, S.C. (1999) Brain Res. 839, 221–226.
[36] Fazekas, F., Kapeller, P., Schmidt, R., Offenbacher, H., Payer, F. and Fazekas, G. (1996) J. Neurol. Sci. 142, 121–125.
[37] Imon, Y., Yamaguchi, S., Yamamura, Y., Tsuji, S., Kajima, T. and Ito, K. (1995) J. Neurol. Sci. 134, 27–32.
[38] Parsey, R.V. and Krishnan, K.R.R. (1998) Psychiat. Res. Neuroimag. 82, 181–185.
[39] Gorrell, J.M., Ordidge, R.J., Brown, G.G., Deniau, J.-C., Buderer, N.M. and Helpern, J.A. (1995) Neurology 45, 1138–1143.
[40] Chen, J., Hardy, P.A., Clauberg, M., Joshi, J.G. and Parravano, J. (1989) Radiology 173, 521–526.
[41] Sjøgren, C.E., Briley-Saebø, K., Hanson, M. and Johansson, C. (1994) Magn. Reson. Med. 31, 268–272.
[42] Jung, C.W., Rogers, J.M. and Groman, E.V. (1999) J. Magn. Magn. Mat. 194, 210–216.
[43] Clark, P.R. and St. Pierre, T.G. (2000) Magn. Res. Imag. 18, 431–438.