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Histological Co-Localization of Iron in Aβ Plaques of PS/APP Transgenic Mice

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Abstract

This study confirms the presence of iron, co-localized with Aβ plaques, in PS/APP mouse brain, using Perls' stain for Fe^{3+} supplemented by 3,3′-diaminobenzidine (DAB) and Aβ immunohistochemistry in histological brains sections fixed with formalin or methacarn. In this study, the fixation process and the slice thickness did not interfere with the Perls' technique. The presence of iron in β-amyloid plaques in PS/APP transgenic mice, a model of Alzheimer's disease (AD) pathology, may explain previous reports of reductions of transverse relaxation time (T_2) in MRI studies and represent the source of the intrinsic Aβ plaque MR contrast in this model.

Keywords

β-amyloid; Alzheimer's disease; Brain; Iron; MRI; Transgenic mice

INTRODUCTION

Iron is important for normal brain development and for most of the critical metabolic functions, including energy metabolism and synthesis of neurotransmitters. Brain iron increases with age and different cerebral regions accumulate iron at different rates and concentrations (1–3). The highest concentrations of iron in human adult brains are found in the basal ganglia, more specifically in the globus pallidus, red nucleus and substantia nigra. Iron is also present in white matter, especially during brain development, where it is found in high levels in oligodendrocytes and it is required for myelin production (1).

The blood–brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier control the iron uptake into the brain by regulating the expression of transport proteins' receptors, such as transferrin receptor expression in endothelial and choroids plexus cells (2,4,5). In normal

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brain, iron is not toxic despite its high levels, probably because of efficient homeostatic mechanism. Brain iron is contained in enzymes, in structural proteins, in transport proteins and in storage proteins such as ferritin. If there is an excess of iron or disruption of the homeostasis, iron-induced oxidative damage occurs (6–8).

Brain iron is abnormally elevated in several neurodegenerative disorders, including Alzheimer's disease (AD) (9,10,11), with two times more iron in the neuropil of AD patients than in non-demented patients (12). It is known that Aβ protein has metal-ion-binding sites and that the interaction between Aβ and iron is one of the factors responsible for the aggregation and deposition of Aβ (11,13). The role of iron in the pathogenesis of AD is not completely clear. There are evidence that in binding with iron, Aβ is acting as a chelator to diminish the oxidative damage of high concentrations of iron (7,8), but in the end, the imbalance of the iron metabolism and its accumulation appears to contribute to enhance the oxidative stress implicated as one of the potential mechanisms for the neurodegenerative changes seen in AD.

The presence of iron in and around amyloid plaques in post mortem human brain tissue has been demonstrated using Perls' technique despite the fact that the brain tissue was in general submitted to different concentrations of formaldehyde-based fixation (14,15). Indeed, iron histochemistry using Perls' technique has been the method of choice for non-heme iron detection in tissue sections. Although this is a universally accepted method, its sensitivity and poor penetration on fixed and paraffin embedded tissue has limited the iron detection in certain studies, resulting in controversy about the accuracy of this staining technique. Even with modifications like DAB enhancement (3,3′-diaminobenzidine) introduced to enhances the signal and to improve the sensitivity of the standard Perls' stain (16), there have been conflicting reports about Perls' method not working on formalin-fixed tissue, but only when using tissue fixed in methacarn or fresh unfixed tissue (6). This controversy is also present on studies of the iron distribution in animal brain tissue (1,17,18). Although iron has been demonstrated in formalin fixed mouse (19,20) and rat (21) brain, Smith et al. (18) studying one model of AD transgenic mouse, were able to demonstrate the presence of iron only in brain tissue fixed in methacarn. Despite the vast literature about transgenic mouse models of AD, to our knowledge only the Smith et al. (18) study has attempted to demonstrate, by histochemical methods, the presence of iron in the brain of these transgenic mice. Other studies using biochemical and analytical methods have demonstrated an increase in the level of metals, including iron, in the brain of different mouse models of AD (22,23).

The histological demonstration of iron in Aβ plaques is important not only because of its possible role in the pathogenesis of the disease, but also because Aβ-iron complex could be used as a mechanism of detection and progression of the disease by MRI – histology correlation studies. Our laboratory and others have investigated iron in association with reduced T_2 in MRI studies of normal and diseased human brain (24–26). Recently, using high resolution MRI, we have reported reduced mean T_2 values in the hippocampus and cortex of 18 months old PS/APP mice *in vivo* (27) and detected Aβ plaques *in vitro* without the use of any contrast reagents (28). The PS/APP transgenic mouse is a model of Alzheimer pathology overexpressing both mutante human APP and PS1, and showing an early and extensive amyloid deposition. We hypothesize that the presence of iron in Aβ plaques, in this model, is the significant factor responsible for reducing T_2 and the source of the plaque intrinsic MRI contrast seen in these animals. The objective of the present study is to investigate the presence of iron in Aβ plaques of the transgenic mouse model of Alzheimer pathology (PS/APP), in which we have previously demonstrated changes in MRI parameters. Additionally, this study also addresses the question of the role of formalin fixation on the accuracy of the Perls' staining.

EXPERIMENTAL PROCEDURE

Three 18-month-old PS/APP transgenic mice and one age-matched non-transgenic (NTg) control mouse were used. The PS/APP transgenic mouse is a model of Alzheimer pathology over-expressing both mutante human APP and PS1, and showing an early and extensive amyloid deposition (29).

For histochemical analysis, two PS/APP and one NTg were perfused with phosphate-buffered saline (PBS) (pH 7.4) through the left cardiac ventricle, followed by 10% buffered formalin. After perfusion fixation, the brains were removed and stored in 10% buffered formalin at room temperature. A third 18-month-old PS/APP mouse, after PBS perfusion, had the brain fixed in methacarn as described by Smith et al. (6). After fixation, one PS/APP mouse brain fixed in formalin, one fixed in methacarn and the NTg mouse brain, were then cut serially in 40 μm-thick coronal sections using a vibrotome (series 1000). The other PS/APP mouse brain fixed in formalin was cut in 20 μm-thick coronal sections. To show the presence of iron in Aβ plaques, neighboring brain sections were staining with either Aβ antibody or DAB-enhanced Perls' reaction and counterstained with nuclear fast red. The Aβ immunohistochemistry was performed using antibody 6E10 at 1:1000 (Sigma, St. Louis, MO, USA). Sections were immunostained by a standard avidin–biotin complex method using diaminobenzidine as chromogen. The Perls' reaction following 3,3-diaminobenzidine (DAB) enhancement (16) staining is based on the formation of ferric ferrocyanide (Prussian blue) when ferric ion (Fe^{3+}), released from iron-containing compounds by hydrochloric acid (HCl), reacts with potassium ferrocyanide. The ferric ferrocyanide then catalyzes the oxidation of DAB with formation of a brown precipitate.

RESULTS

The presence of iron in brain sections of all mice was observed, stained with similar intensity in both transgenic and NTg mice cells in and around white matter tracks at the level of the Caudate-Putamen (Fig. 1a, b). Additionally, histological sections fixed with formalin (Fig. 1c) or methacarn (Fig.1d) from PS/APP mice brains showed the presence of iron in the majority of Aβ plaques. A similar pattern of distribution of Aβ plaques and iron staining could be seen in these mice brains (Fig. 1e, f), which serves to support the co-localization of iron in Aβ plaques. There was no positive staining for Aβ plaques in the negative control sections or sections stained only with DAB, indicating the specificity of the staining method.

DISCUSSION

Despite the fact that the Perls' technique is routinely used for histological detection of iron in tissue, a controversy about the accuracy and sensitivity of this staining technique exist. There are reports that this histochemical technique does not work or is greatly reduced by formalin fixation and will only work on frozen or tissue fixed in methacarn, mainly in studies aimed at detecting the presence of iron in amyloid deposits (6,18). Therefore, the histological confirmation of Aβ-iron complex in tissue fixed with different fixatives was important, particularly for many MRI – histology correlation studies.

We have demonstrated the presence of iron in PS/APP mouse brains using Perls' stain for Fe^{3+} supplemented by 3,3′-diaminobenzidine (+DAB). Also, since formaldehyde-based fixation is supposedly considered to interfere with the iron localization by reducing the labeling, we tested two PS/APP mice brains, one fixed with methacarn and one fixed with formalin, and despite the fixation method, both brains showed the presence of Fe^{3+} in

amyloid plaques. Thus, the fixation process and the slice thickness did not appear to interfere with the Perls' technique, at least in our study.

The observation of iron accumulation in Aβ plaques in PS/APP mice is consistent with previous studies in humans (15,14), and helps to clarify the controversy about the accuracy of this staining technique in mice brain tissue. Despite the vast literature about transgenic mouse models of AD, this is the first study to demonstrate the co-localization of iron in Aβ plaques in PS/APP mouse brains using Perls'+DAB method.

The presence of iron in Aβ plaques in this transgenic mouse model of AD pathology confirms the source of the plaque intrinsic MRI contrast in these animals. In particular, this work supports our hypothesis that the presence of iron in the Aβ plaques is a major factor in the signal hypo-intensity seen in our in vitro images (28) and in the reduction of T_2 measured *in vivo* (27) in PS/APP mouse brain. Additionally, the presence of iron could possibly be exploited as an endogenous contrast reagent utilizing new MRI techniques designed to be contrast sensitive to the presence of microscopic susceptibility effects (30), which will be important in future studies that aim to correlate different quantification indices applied to both MRI and histological images. We acknowledge that further work will be necessary to also investigate the presence of Fe^{2+} and ferritin in these transgenic mice, which would allow us to have a more complete view of the relationship between the effects of the imbalance of the iron metabolism and the MRI measurable parameters.

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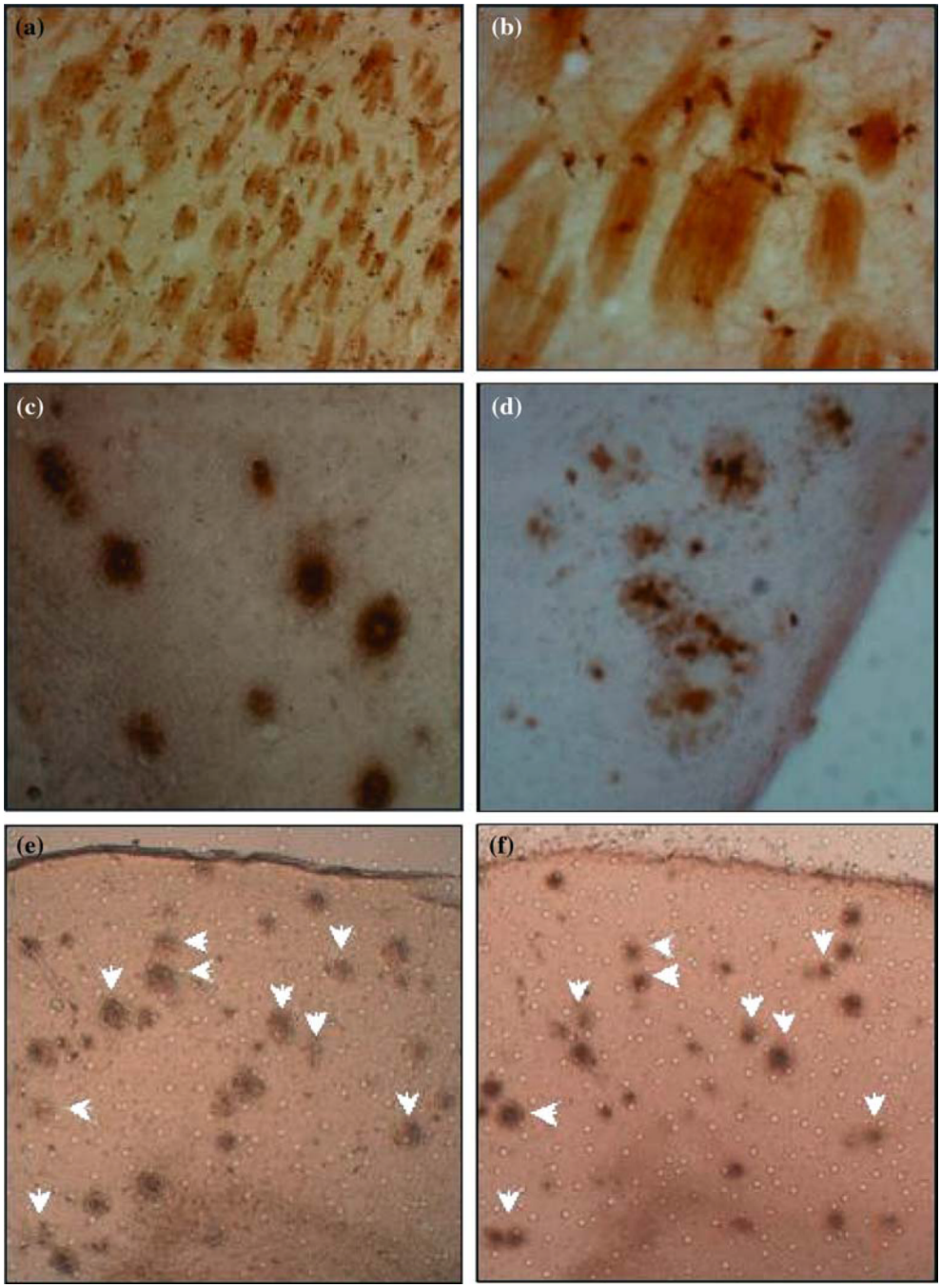

Fig. 1.

Low (a) and High-power (b) view of histological sections from Caudate-Putamen showing the presence of Fe^{3+} in cells in and around white matter tracks; High-power view showing Fe^{3+} in amyloid plaques of transgenic PS/APP mice detected by Perl's reaction followed by DAB enhancement in brains fixed with formalin (c) and methacarn (d). A similar pattern of distribution of Aβ plaques and iron staining was seen in neighboring brain sections (e and f, white arrows), which confirmed the co-localization of iron in Aβ plaques.