BioFactors

Oligodendrogenesis: The role of iron

*Maria Elvira Badaracco, Maria Victoria Rosato Siri, Juana Maria Pasquini**

Facultad de Farmacia y Bioquímica, Departamento de Química Biológica, Instituto de Química y Fisicoquímica Biológica (IQUIFIB), UBA-CONICET, Buenos Aires, Argentina

Abstract.

Iron seems to be an essential factor in myelination and oligodendrocyte (OLGc) biology. However, the specific role of iron in these processes remains to be elucidated. Iron deficiency (ID) imposed to developing rats has been a relevant model to understand the role of iron in oligodendrogenesis and myelination. During early development ID causes specific changes in myelin composition, including a lower relative content of cholesterol, proteolipid protein (PLP), and myelin basic protein 21 (MBP21). These changes could be a consequence of the adverse effects of ID on OLGc development and function. We subsenquently studied the possible corrective effect of a single intracranial injection (ICI) of apotransferrin (aTf) on myelin formation in ID rats OLGc migration and differentiation after an ICI of aTf was evaluated at 3 days of age. ID increased the number of proliferating and undifferentiated cells in the corpus callosum (CC), while a single aTf injection reverts these effects, increasing the number of mature cells and myelin formation. Overall, results of a series of studies supports the concept that iron may affect OLGc development at early stages of embryogenesis rather than during late development. Myelin composition is altered by a limited iron supply, changes that can be reverted by a single injection of aTf.

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E-mail: jpasquin@qb.ffyb.uba.ar

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1. Introduction

Iron deficiency (ID) is a common nutritional disorder, particularly among children, affecting more than 25% of the population around the world [1]. Children who experienced ID suffer behavioral defects [2]. A close correlation between ID and brain function has been also observed in rodent models. In this regard, ID is in general associated with important changes in neurotransmission and myelination [3].

The oligodendrocyte (OLGc) is the cell type with highest iron content in the brain [4]. During development, brain iron and ferritin are initially present in microglia, and subsequently, when myelination is initiated, iron, ferritin, and transferrin are found in OLGc.

Different proteins and enzymes utilize iron as a cofactor. In addition, mitochondria require iron for its function and a great number of proteins such as respiratory chain complexes use iron-sulfur cluster as cofactors [5]. The central nervous system (CNS) has high iron content, and the mechanisms participating in brain iron uptake and homeostasis are of high interest for all researchers working in this area. Brain iron uptake is high in infant brain during development, given that the blood brain barrier (BBB) is not yet fully formed [6]. Recent studies indicate that Tf receptors (TfR) are present at the luminal membranes of capillary endothelial cells and even though Tf does not cross endothelial cells, iron can enter into the brain interstitium [7].

Our hypothesis is that events involving iron uptake and homeostasis by OLGc are critical to myelin composition and formation, and their disruption can significantly affect myelination. However treatment of ID animals with apotransferrin (aTf) causes an accelerated OLGc differentiation and myelin formation by a mechanism not involving the iron transport but producing a significant correction in the different parameters affected by the iron absence.

2. Myelin composition of adult iron-deficient rats

Dietary ID during development was produced as follows: pregnant Wistar dams were fed a control diet (40 mg Fe/kg) or an iron deficient (ID) diet (4 mg Fe/kg) beginning at gestational day 5 and until day 20, returned to a normal diet and studied at 6 months of age; it was associated with less total myelin protein as well as decreased cholesterol, phospholipids, and galactolipids content [8]. The largest change

*Address for correspondence: Juana Maria Pasquini, Ph.D., Facultad de Farmacia y Bioquímica, Departamento de Química Biológica, Instituto de Química y Fisicoquímica Biológica (IQUIFIB), UBA-CONICET, Junín 956, Buenos Aires C1113AAD, Argentina. phone: +54-11-49648287 Fax: +54-11-49625457; E-mail: jpasquin@qb.ffyb.uba.ar.
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observed in these animals was a 30% decrease in cholesterol. In the same rats, the protein profile of myelin revealed that the proteolipid protein (PLP) and the myelin basic protein 21 (MBP21) declined as a percentage of the total myelin protein (Fig. 1). All of the integral myelin proteins, except for CNPase and MBP14, decreased when referred to the weight of the tissue. The fatty acid composition was similar between the two groups, except for fatty acid 20:4. The relative amounts of transferrin (Tf) and ferritin in the myelin fraction of the ID rats showed that Tf decreased by over 50% when compared to control rats. On the other hand, ferritin levels were similar between the two groups. In myelin iron, concentrations were similar for ID and control rats. Similarly to the case for the myelin fraction, there was no difference in the iron concentration between ID and control rats in the different subcellular fractions. A decreased iron availability affected both the quantity and the quality of myelin. Myelin composition and quantity were altered by ID even when the myelin iron content was not affected. One of the important points of this analysis was to determine whether there were specific deficits within myelin composition associated with specific disruptions in iron homeostasis. The decrease in myelin protein content associated with developmental dietary ID was fairly uniform, while PLP and MBP21 decreased as a percentage of the total protein. PLP is required for compaction and formation of myelin sheaths, and may be required for OLGc maturation [9]; MBP21 is part of a family of intracellular adhesion proteins [10] which expression pattern in myelin remains unchanged with age [11]. The decrease in PLP and MBP21 relative content suggests that the compaction of myelin is affected in ID. Furthermore, an attempt to correct developmental ID by a return to normal dietary iron levels at weaning, normalizes myelin iron levels but does not reestablish normal myelin composition.

Iron is a structural component of both delta 6 and delta 9 desaturase enzymes [12,13], and delta 9 desaturase has been shown to be reduced as a result of dietary ID [14]. Severe dietary iron restriction produced significant changes in brain fatty acid composition [15,16]. A feeding paradigm in mice, similar to ours, resulted in a decrease in fatty acid composition [17]. Only the 20:0 fatty acid family was affected in studies carried out by our group [8], while in a more severe model of dietary iron restriction in rats, the sum of the n-3 fatty acids was decreased, but not the n-6. The only fatty acid family that decreased with ID was 20:4 [18].

3. Oligodendroglial cell analysis and myelin composition of ID rats at developmental ages

Using the same model of rat ID mentioned before, the effects of a decreased iron availability on OLGc maturation and myelinogenesis at postnatal days 17 (P17) and 24 (P24) were investigated. The possible beneficial influence of an intracranial injection (ICI) of aTf at 3 days of age in ID rats was also investigated [19]. Administration of an ID diet to

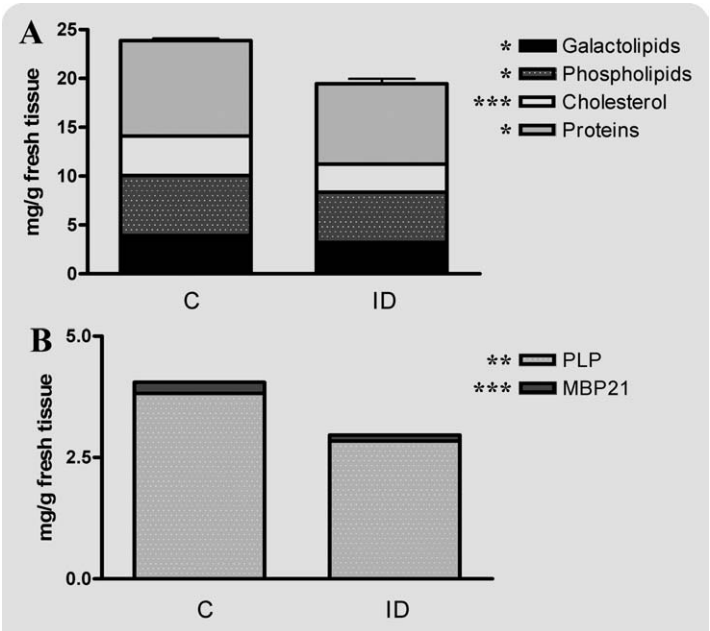

Fig. 1. Myelin composition of adult iron-deficient rats. (A) Myelin composition is expressed as mg/g fresh tissue (mean ± SEM). (B) Proteolipid protein (PLP) and isoform 21 of myelin basic protein (MBP21) as a percentage of total protein. Values are expressed as mg protein/g fresh tissue (*$P < 0.05$, **$P < 0.01$, *** $P < 0.001$).

pregnant dams during gestation induced a marked decrease in the offspring's brain weight, which was evident at 24 days of age and could be a result of a reduced accumulation of myelin membranes. At P24, ID caused a decrease in myelin proteins and lipids. In ID animals, we observed a 50% decrease in myelin dry weight/g of fresh tissue compared to controls. Myelin cholesterol, phospholipids, and galactolipids, as well as myelin proteins were markedly reduced by ID, supporting the conclusion that myelination in ID rats is severely affected. Except for cholesterol, which was significantly lower in 17-day-old rats, no significant changes in myelin composition were detected at this age. Cholesterol could have been affected by ID at earlier stages of development, probably because its synthesis is highly dependent on the availability of normal amounts of this trace element. An increase in cholesterol content was observed when the ID rats were ICI with aTf at 3 days of age. These results indicate that the effect of exogenous aTf on the myelination process in ID rats could be similar to what occurs in normal young rats [20,21]. That is, aTf stimulates OLGc maturation and remyelination, and even in the absence of a normal iron supply, aTf produces a significant correction in the different biochemical parameters affected by ID. The myelin membrane is a unique membrane in the aspect that it contains a high lipid/protein ratio and is enriched in glycosphingolipids and cholesterol [22]. Lipid raft microdomains were conceived as part of a mechanism for the intracellular trafficking of lipids and lipid-anchored proteins. Lipid rafts in cell membranes are defined by their detergent solubility and by their

sensitivity to cholesterol deprivation. Studies were done in primary OLGc cultures from ID rats and compared with OLGc cultures prepared from normal controls or from ID animals treated with aTf. PrPc (protein prion cellular) is a protein attached to Triton X-100-insoluble, low-density complexes or rafts and used as raft marker. The immunoreactivity of PrPc in OLGc cultures from ID rats is lower than that in controls, which indicates that in these cells, lipid raft formation must be defective. Because these structures are known to be of fundamental importance in the formation of myelin [23], it is tempting to conclude that a major reason for hypomyelination in ID rats could be an abnormality in raft formation as a result of a decrease in cholesterol.

Although it has been shown that almost 80% of iron in rat brains are found in the myelin fraction [24]; there is limited information regarding the presence of Tf in myelin. A previous study by Chen et al. [25] showed that early postnatal weaning in iron deficient rats induces a marked increase in transferrin in a microsomal fraction obtained from the brain. Although these results were obtained in a different subcellular fraction, they agree with those described above. This increase in Tf was considered by these investigators to be the consequence of an adaptive mechanism to meet the iron requirements of brain growth in a situation of impaired iron availability. If the interpretation of Chen et al. [25] is correct, it could be speculated that in our ID model, the increase in Tf in the myelin fraction could be the consequence of a fully operating adaptive response. A recent publication [26] gives strong support to the hypothesis of a synergistic relationship between microglia and oligodendrocytes that can be modified by iron status. These investigators clearly show that ferritin, released by microglia, is an important source of iron for oligodendrocytes. The ability of oligodendrocytes to bind and internalize ferritin, specifically the H subunit of ferritin, has been previously demonstrated [27]. The binding of H-ferritin to white matter tracts [28], has also been shown to be coincident with the onset of myelination [29]. Badaracco et al. [19] showed that in the corpus callosum (CC) of the ID rats, H-ferritin immunoreactivity was greatly diminished. This decrease, however, appears to have been compensated to a large extent in the animals that received an ICI of a single dose of aTf. Recently, Moos and Morgan [30] demonstrated that dietary ID alters the cellular content of transferrin and ferritin receptors, ID caused a higher expression of transferrin and a decreased ferritin receptor expression (Fig. 2).

Coincident with these results, in our laboratories we have found that the immunoreactivity of transferrin in myelin membranes isolated from ID rats was higher than normal, whereas the immunoreactivity of ferritin in the CC of ID animals was lower than normal. It is well known that iron is predominantly localized in OLGc. Sanyal et al. [31] showed that the expression of H-chain ferritin is up-regulated during OLGc regeneration/differentiation. This agrees with results showing that in different types of cells, ferritin transcription is modulated by molecules that control cell growth and differentiation. The transcription takes place on OLGc–substratum adhesion and can be viewed as another manifestation

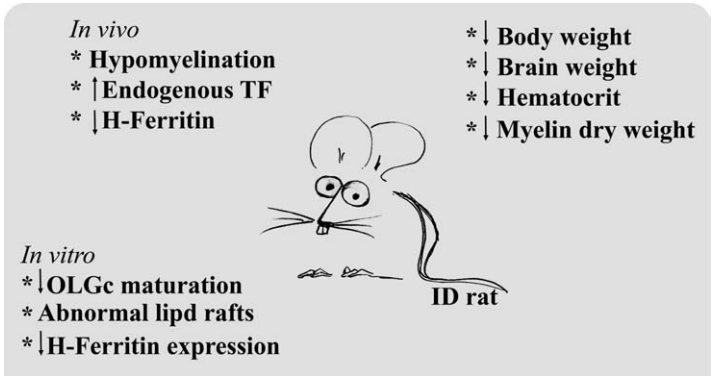

Fig. 2. This figure summaries a set of experimental data obtained in the ID animal. In vivo and in vitro experiments were performed to characterize iron deficiency effect on either developmental parameters and myelin integrity and composition. See text for further details. Tf, transferrin; OLGc, oligodendroglial cell; ID, iron deficiency.

of the anchorage-induced signal that finally ends in the differentiation of OLGcs. In OLGc cultures from ID rats, we showed a decreased level of ferritin that could explain why these cells were not able to differentiate in culture (Fig. 2).

4. Oligodendrocytes: migration and differentiation

We also investigated the behavior of OLG precursor cells in the subventricular zone (SVZ) and the CC of rats submitted to an ID diet from birth. Studies were done at P3 and P11 and involved an immunohistochemical analysis of cell proliferation using BrdU labeling, a characterization of OLGcs and their precursors and an analysis of their dynamics (Fig. 3). A critical finding of this work is that a single ICI of aTf ameliorates the adverse effects of ID.

Cell population at P3 was first studied since, at this age, the ICI of aTf can induce an increased maturation of the OLGc precursors [32]. At this early age, the relative amount of PCNA positive (proliferating) cells was slightly higher in ID compared to controls. The population of doubly marked cells (PCNA/Olig1 or PCNA/O4) in the IDs also increased, indicating that most of the PCNA positive cells remained in an undifferentiated state as opposed to control animals. These results suggest that the availability of undifferentiated precursors was higher in the ID group. One possibility is that in ID animals the OLGc precursors were unable to transit along the different OLGc maturation stages beyond the OPC (oligo-precursor cells) level and that, consequently, they could not synthesize a normal, mature myelin. These results are in agreement with those of Morath and Mayer-Proschel [33], who described that during pregnancy ID affects the levels of brain iron in the developing fetus and not only disrupts the proliferation of their glial precursor cells, but also disturbs the generation of oligodendrocytes from these precursor cells.

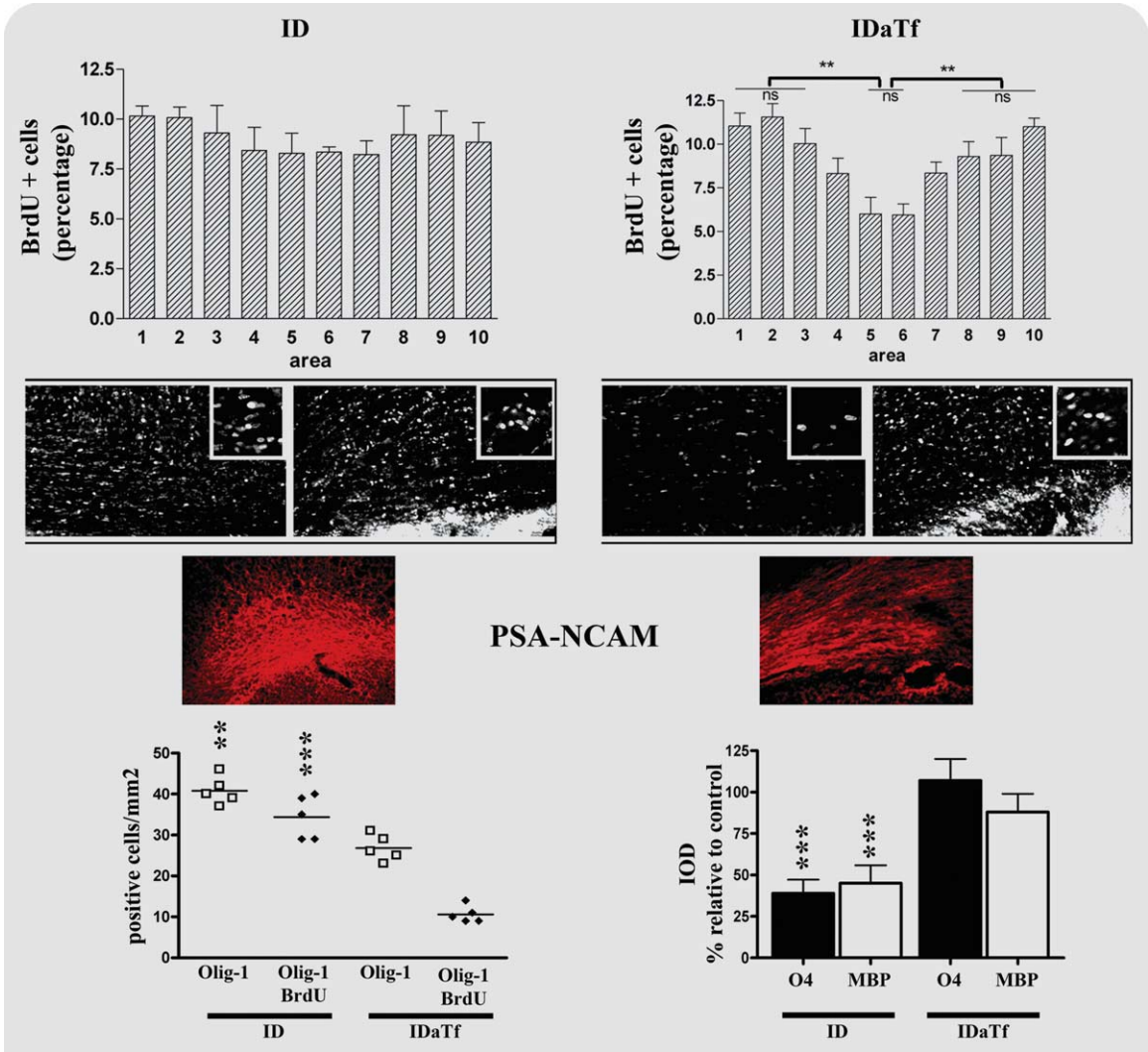

Fig. 3. Oligodendroglial cell migration and differentiation. Quantitative analysis and distribution of BrdU positive cells in the corpus callosum (CC). Distribution of BrdU positives cells is expressed as the percentage of cells in different areas of the CC for ID and IDaTf animals. Representative dark-field images of: medial CC (left) and lateral CC (right) of each group are shown. To evaluate cells in the process of active migration, we analyzed cells expressing PSA-NCAM. Values are the mean of positive cells/mm² or mean IOD/mm² and expressed as a percentage of control conditions ($P < 0.01$, ***$P < 0.001$, ns: not significant). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

Cell population at P11 after a BrdU injection at P4 showed that the BrdU+ cells are increased in ID animals as compared to controls and reverted to a decreased population after the ICI of aTf. In coincidence with our results, Morath and Mayer Proschel [33] demonstrated that at E 13.5 there is a slight but not statistically significant increase in the proportion of BrdU+/A2B5+ cells in the spinal cord. In coincidence with our results, iron deprivation in HL-60 cells blocked phorbol myristate acetate-induced cell differentiation by inhibiting the induction of the cell cyclin-dependent kinase inhibitor p21, releasing cells from the G1/S cell cycle checkpoint [34].

We observed that, as a consequence of enhanced oligodendrogenesis and decreased oligodendrocyte maturation in ID, the number of myelinated axons present at P11 is decreased, as evaluated by a double immunostaining with anti MBP and neurofilament-200 (NFH) antibodies. After a single ICI of aTf, the number of myelinated axons returned to control values. These results correlate well with electrophysiological measurements. Since the optic nerve is an important structure, rich in oligodendrocytes which can be affected by ID, we decided to compare our immunohistochemical observations with electrophysiological mesurements. Recording of visual evoked potentials (VEPs) showed a defective response to visual stimuli in ID animals compared to control rats. When ID rats were ICI with aTf, the electrophysiological assays confirmed once again that the optic nerve response to the visual stimuli became similar to those of controls.

Our results are in coincidence with those of Morath and Mayer-Proschel [33] and raise the possibility that iron may affect OLGc development at early stages of

embryogenesis rather than during late development. Results also give support to the hypothesis that ID increases the number of proliferating and undifferentiated cells in the CC, while a single injection of aTf reverts this effect increasing the number of mature cells and the deposition of myelin.

ID animals which exhibit an enlarged undifferentiated cell subpopulation able to migrate have a limited capacity to progress beyond the precursor stage. The OPCs population might recover the capacity for maturation and myelination as a consequence of the ICI of aTf. The above evidence supports the hypothesis that aTf ICI triggers an active process to facilitate myelin repair.

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