CHAPTER I

INTRODUCTION

1. Background and Rationale

Iron is a required cofactor of many key enzymes involved in energy metabolism, cholesterol, lipid, and DNA syntheses. At the cellular level, iron is bound to and mobilized by iron transport protein, transferrin (Tf). Expression of its putative receptor is ubiquitously found on most cell types with highest levels observed in normal proliferating cells, including myelin-forming cells of the central nervous system, oligodendrocytes (Ponka and Lok, 1999; Lin and Connor, 1989). Oligodendrocytes express transferrin receptor (TfR) during the initial stages of their development before galactocerebrosides, Tf and other myelin markers (Espinosa de los Monteros et al., 1988; Lin and Connor, 1989). It is now established that mature oligodendrocytes are the principal iron-containing cells with high expression levels of Tf (Dickinson and Connor, 1995; Benkovic and Connor, 1993). Alteration in iron acquisition pattern during postnatal development induced by prenatal ethanol exposure was associated with delayed onset of myelination and, subsequently, hypomyelination of white matter tracts, suggesting iron is an important factor for oligodendrocyte myelination (Connor and Menzies, 1996). Furthermore, even with aggressive replacement of Tf in the adult hypotransferrinemic mouse, a mutant that carries a splicing defect in the Tf gene with a phenotype of < 1% of circulating levels of Tf, is hypomyelinated (Dickinson and Connor, 1994). Taken together, these data indicate that iron-saturated Tf plays a critical role in cellular iron uptake, growth and differentiation of myelinating oligodendrocytes.

On the other hand, our knowledge on the relationship of iron to Schwann cells and myelination is still limited. Evidence has provided that axotomy led to a massive but transient increase in Tf binding at the site of the injury and in the distal part of the

crushed or resected sciatic nerve, shortly preceding the time course of Schwann cell proliferation. Immunocytochemistry revealed strong and simultaneous expression of the TfR on two different cell types: invading macrophages and Schwann cells reacting to the injury (Raivich et al., 1991). These observations lead us to hypothesize that activation of Schwann cell iron metabolism could be essential for the process of nerve regeneration and could recapitulate relationship of iron to oligodendrocytes and myelination. Therefore, in this study aim to examine the effect of iron on proliferation and myelin-associated differentiation of Schwann cells and hypothesized that iron could influence Schwann cell proliferation and/or differentiation.

2. Research Questions

Primary Question

Does iron exposure influence Schwann cell proliferation and differentiation?

Secondary Question

Does iron exposure influence transferrin receptor (TfR) and ferritin, iron storage protein expression in Schwann cells?

3. Research objectives

1. To examine the proliferative effect of iron on cultured Schwann cells using MTT proliferation/ cytotoxic assay and trypan blue dye exclusion assay

2. To investigate the effect of iron on TfR expression in cultured Schwann cells using flow cytometry assay

3. To examine the effect of iron on the expression of iron storage protein ferritin in cultured Schwann cells using western blot analysis

4. To determine the effect of iron on the mRNA expression of myelin-markers (P0 and MBP) associated with Schwann cell differentiation using RT-PCR assay

4. Hypothesis

Iron exposure could induce Schwann cell proliferation. It is also speculated that iron at high concentration could induce the expression of myelin-markers associated with Schwann cell differentiation.

5. Keywords

Iron

Transferrin receptor

Ferritin

Myelin protein zero

Myelin basic protein

6. Expected Benefits & Applications

Results from this study will establish relationship of iron to Schwann cell and PNS myelination. Moreover, understanding factors influencing Schwann cell differentiation could be useful in therapeutic use of Schwann cell for myelin repair in autoimmune demyelinating disorders.