CHAPTER V

DISCUSSION AND CONCLUSIONS

One of the important events in the development of nervous system is the differentiation of myelin making cells, the CNS oligodendrocytes and the PNS Schwann cells. The several studies indicated that iron is absolutely required for oligodendrocyte myelination (Ponka and Lok, 1999; Lin and Connor, 1989). During the initial stage of their development oligodendrocytes express TfR before GalC and MBP (Espinosa de los Monteros et al., 1988; Lin and Connor, 1989). Alteration in iron acquisition during postnatal development induced by prenatal ethanol exposure was associated with delayed onset of myelination and hypomyelination of white matter tracts (Connor and Menzies, 1996). On the other hand, the relationship of iron and Schwann cells has not been elucidated. Therefore, the present study was designed to examine the effects of iron on Schwann cells proliferation and differentiation. Moreover, this study intented to investigate the mechanisms involved in iron regulation in this cell type.

The Schwann D6P2T cells, derived from a N-ethyl-N-nitrosourea induced rat peripheral neurotumor, was selected as an *in vitro* model for this study. This particular cell line possesses the characteristic of immature Schwann cells, which can be isolated from sciatic nerves neonatal rats (Muja et al., 2001). Morphologically, the D6P2T cells have a spindle shape resemble primary Schwann cells in cultures (Bunge et al, 1983). They express S100 protein which is a marker of immature cells (Hai et al, 2002). Moreover, it has been shown that the cell line can express protein and transcripts of P0, transcripts of PMP22 and CNPase protein (Bansal and Pfeiffer, 1987). The latter is a myelin enzyme involved in ion transport (Ledeen, 1992). All together, this cell line is therefore suitable for the purpose of this study.

Previously, it has been shown that the D6P2T cells expressed P0 and PMP22 genes when cultured in 10% serum containing medium (Hai et al, 2002), but they do

not express other myelin genes such as MBP and MAG. In this study, we were able to induce the expression of MBP transcripts along with that of P0 by reducing serum concentration from 10% to 1% serum containing medium. Therefore, we used 1% serum containing medium throughout the study to examine the effect of iron on Schwann cell proliferation and differentiation.

Iron in the form of ferric ammonium citrate was used to facilitate iron entry into the cells as previously reported (Parkes et al., 1993; Hirsh et al., 2002). The concentrations of iron citrate range from 50-1000 μg/ml, which overlaps the range of concentrations that Schwann cells might be encountered under physiological and pathological conditions; 100 and 300 μg/ml, respectively (Rogers and Lahiri, 2004).

As shown by MTT assay, exposure of Schwann cells to various concentrations of iron yielded different outcome. A low concentrations of either 50 or 100 μg/ml induced Schwann cell proliferation. This result was confirmed by a direct count of viable cells excluded from trypan blue dye. An increase in Schwann cell proliferation may be associated with elevated levels of intracellular iron reflected by alterations in expression of iron regulatory proteins; TfR and ferritin which known to be regulated at post-transcriptional level (Theil, 1994; Klausner, 1993; Hentze, 1996). This amount of iron could facilitate the action of ribonucleotide reductase, which is a rate-limiting enzyme in DNA synthesis (Fairbanks, 1999; Beard and Dawson, 1996; Liu and Templeton, 2006), before the excess was put into storage in ferritin (Crichton et al., 2002). It is also observed that high concentrations of iron, when applied to cultured Schwann cells, stimulated cell proliferation in a lesser extent without being toxic. It could be explained that elevation of intracellular iron following an exposure to high concentration of iron may induce Schwann cell differentiation. This explanation is supported by the fact that Schwann cells in early stage of myelination display a very low rate of cell proliferation (Gandelman et al, 1989). Furthermore, a decrease in ferritin expression in response to high concentration of iron suggested that iron could

be used possibly by different enzymes that require iron as a cofactor, including those involved in lipid and myelin synthesis (Larkin and Rao, 1990).

Interestingly, it was found that exposure to iron induced transcript expression of P0 and MBP in a dose-dependent manner. These findings are in agreement with the notion that exposure to high concentration of iron could induce Schwann cell differentiation. The mechanism underlying the effect of iron may be involved a set of transcription factors including Sp1 and NF-kB, which recently are shown that the activation of these transcription factors promote the synthesis of MBP transcripts (Farina et al., 1999; Xiong et al., 2003).

In conclusion, the present study demonstrates that the role of iron in Schwann cell development, in which iron could exert its effect on cell proliferation and differentiation depending upon its concentration available (Table 4). These finding may be useful application in therapeutic use of Schwann cells for myelin repair in autoimmune demyelinating disorders, such as MS in which iron deposition has been reported in the white matter lesions (Craelius et al., 1982).

Table 4 Mitogenic and differentiative effects of iron on Schwann cells

Iron concentration (μg/ml) / Effects of iron on Schwann cells		Low concentration		High concentration		
		50	100	250	500	1000
I. Proliferation		5+	4+	3+	2+	1+
II. Iron metabolism	TfR	Decreased about 50% of untreated control				
	Ferritin	4+	5+	3+	2+	1+
III. Differentiation (transcript levels)	P0	1+	2+	3+	4+	5+
	MBP	1+	2+	3+	4+	5+

+ Increasing when compared with D6P2T untreated control