IJMS
Vol 40, No 6, November 2015

Original Article

Progesterone Enhanced Remyelination in the Mouse Corpus Callosum after Cuprizone Induced Demyelination

Iraj Ragerdi Kashani[1], PhD;
Azim Hedayatpour[1], PhD;
Parichehr Pasbakhsh[1], PhD;
Laya Kafami[2,3], PhD;
Behzad Khallaghi[3], MSc;
Fatemeh Malek[1], MSc

Abstract

Background: Progesterone as a sex steroid hormone is thought to affect and prevent demyelination, but its role in promoting myelin repair is far less investigated. In this study, remyelinating potential of progesterone in corpus callosum was evaluated on an experimental model of MS.

Methods: In this experimental study, adult male C57BL/6 mice were fed with 0.2% (w/w) cuprizone in ground breeder chow ad libitum for 6 weeks. At day zero, after cuprizone removal, mice were divided randomly into two groups: (a) placebo group, which received saline pellet implant, (b) progesterone group, which received progesterone pellet implant. Some mice of the same age were fed with their normal diet to serve as the healthy control group. Two weeks after progesterone administration, Myelin content was assessed by Luxol-fast blue staining. The myelin basic protein (MBP) and proteolipid protein (PLP) expression were assessed using Western blot analysis and the changes in the number of oligodendrocytes and oligodendroglial progenitor cells were assessed by immunohistochemistry (IHC) and flow cytometry.

Results: Luxol-fast blue staining revealed enhanced remyelination in the progesterone group when compared with the placebo group. Densitometry measurements of immunoblots demonstrated that MBP and PLP proteins contents were significantly increased in the progesterone group compared with the placebo group. Flow cytometry and IHC analysis showed increases in Olig2 and O4 cells in the progesterone group compared with the placebo group.

Conclusion: Overall, our results indicate that progesterone treatment can stimulate myelin production and that it may provide a feasible and practical way for remyelination in diseases such as multiple sclerosis.

Please cite this article as: Kashani IR, Hedayatpour A, Pasbakhsh P, Kafami L, Khallaghi B, Malek F. Progesterone Enhanced Remyelination in the Mouse Corpus Callosum after Cuprizone Induced Demyelination. Iran J Med Sci. 2015;40(6):507-514.

Keywords ● Multiple sclerosis ● Remyelination ● Progesterone

[1]Department of Anatomical Sciences, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran;
[2]Department of Pathobiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran;
[3]Shefa Neurosciences Research Center, Tehran, Iran

Correspondence:
Iraj Ragerdi Kashani, PhD;
Department of Anatomical Sciences, School of Medicine, Tehran University of Medical Sciences, 16 Azar Street, Poursina Street, Tehran, Iran
Tel: +98 21 64053410
Fax: +98 21 66419072
Email: ragerdi@sina.tums.ac.ir
Received: 19 October 2013
Revised: 15 December 2013
Accepted: 12 January 2014

Introduction

Multiple sclerosis, a chronic and debilitating inflammatory disease of the CNS is characterized by myelin damage.[1] In multiple sclerosis, although resident oligodendrocyte progenitors are found around the lesions, they remain in a quiescent state and remyelination is incomplete.[2] Thus, therapeutic strategies that

promote remyelination are likely to have significant beneficial effects. One approach is to enhance or re-activate the endogenous process.[3] The idea that sex steroids are involved in MS and that they might be a therapeutic option also originates from retrospective studies revealing that pregnancy has a favorable trend for the course of MS on a short- and long-term basis.[4] Remission of symptoms is seen during pregnancy, in particular during the last trimester when estrogens and progesterone plasma levels are at their maximum.[5] Postpartum, MS symptoms exacerbate again.[4] Recent reports, however, have shown that progesterone decreases neuropathology when given at the time of demyelination induction,[1,3,6] but its therapeutic role in promoting myelin repair after induced demyelination is far less investigated.[7-10] This is due to the conflicting results obtained in the experimental autoimmune encephalomyelitis (EAE) model, which indicated negative as well as positive effects for progesterone on disease progression.[7,8]

In vivo studies on animal models of MS have drawn the attention on immune modulatory functions of sex steroids and thus EAE model has been used.[1,7,8]

To be able to distinguish between the immunomodulating effects of progesterone and direct effects on repair capacity of the brain, we used the potential of non-immune driven cuprizone intoxication. Demyelination in the cuprizone model is an early observable event without damaging other CNS cell types; besides oligodendrocytes and without provoking the breakdown of the blood brain barrier and subsequently causing the invasion of lymphocytes.[1] In addition, the cuprizone model shares common features with the earliest phases of MS lesion development as described by Barnett and Prineas.[11] In this study, remyelinating potential of progesterone in corpus callosum was evaluated after cuprizone-induced demyelination as an experimental model of MS.

Materials and Methods

Induction of Demyelination and Hormone Administration

A total of 20 male C57BL/6 mice (Pasteur Institute of Iran) were fed with 0.2% (w/w) cuprizone (Sigma, USA) in ground breeder chow ad libitum for 6 weeks. This diet leads to selective oligodendrocyte death followed by demyelination of axons mainly in the corpus callosum.[12] On day zero after cuprizone removal, animals were randomly divided into two groups: (a) placebo group, which received saline pellet implant (n=10), (b) progesterone group, which received progesterone pellet implant (n=10). Some mice of the same age were fed with their normal diet to serve as healthy control group (*n*=10). On day zero after cuprizone removal, progesterone treated mice were implanted with 14-day release pellets containing 200 mg progesterone (Innovative Research of America, Sarasota, USA). Pellets were implanted subcutaneously in the scapular region behind the neck with a 12-gauge trocar as described by the manufacturer. Placebo mice were implanted with saline pellets (Innovative Research of America, Sarasota, USA). Mice were sacrificed at the end of the second week. The animal experiments were approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

Tissue Preparation

For histological and immunohistochemical studies, mice were intracardially perfused with 4% paraformaldehyde (Merck, Germany) containing picric acid. After overnight postfixation, brains were dissected and processed routinely for paraffin (Merck, Germany) embedding. Then, 10 µm serial, sagital sections were prepared from corpus callosum. Among these serial sections, four representative sections at least 240 µm apart from each other were subject to immunostaining with anti olig2 antibody.

Myelin Staining

To stain for myelin content, tissue sections from mice were treated with Luxol fast blue (LFB, Sigma, USA). Sections were stained overnight in LFP at 56°C and washed in 95% ethanol and distilled water to remove excess blue stain. The color was then differentiated (until white matter was easily distinguishable from gray matter) in lithium carbonate solution (Merck, Germany) for 15 sec, followed by distilled water and three washes of 80% alcohol. Slides were passed through fresh xylene (Merck, Germany) twice, mounted with Entellan (Merck, Germany) and cover slipped.[3] In order to evaluate remyelination in LFB stained sections of cuprizone demyelination of the corpus callosum in the mice that received progesterone, three blinded readers scored LFB stained sections between zero and three. A score of three is equivalent to totally myelinated corpus callosum whereas zero is equivalent to totally demyelinated corpus callosum. A score of one or two corresponds to one-third or two-third fiber myelination of the corpus callosum, respectively.[1]

Western Blotting

Protein extract from corpus callosum were centrifuged at 560 g for 5 min; then stored

at -20°C. To ensure loading of equal amounts during Western blotting, protein concentration of each sample was determined with Bradford assay. Equivalent amounts of total protein extract from each sample were mixed with sample buffer, boiled, and loaded onto SDS polyacrylamide gels. Electrophoretic separation of the extracts was typically performed on 7.5–15% gel. The proteins were then transferred to polyvinylidene (PVDF) (Gibco, CA) and probed with MBP antibody (1:3000 in blocking buffer) (Abcam, USA) and PLP antibody (1:1000 in blocking buffer) (Abcam, USA) for an overnight on shaker in 4°C. Membranes were subsequently rinsed in blocking buffer (4 times/3 min each time) and stained with secondary antibody (1:1000 in blocking buffer) (Abcam, USA) for 1 hour. Then, membranes were washed with TBS Tween-20 blocking buffer (3 times) and the proteins were visualized with diaminobenzidine (DAB) kit (Sigma, USA). The membranes were scanned and the photos were analyzed with image 2 software.

Immunohistochemistry

Ten µm sections from body of corpus callosum were incubated with 10% goat serum (Sigma, USA) for 45 min, and then were incubated overnight with rabbit anti-Olig2 polyclonal antibody (1:2000; Millipore, CA, USA). The next day, sections were incubated with biotinylated secondary antibody (Sigma, USA). This was followed by 1 h incubation at RT with avidin-biotin-peroxidise complex. For visualization, the DAB was used. Finally, the sections were dehydrated in a graded series of ethanol, cleared in xylene, and coverslipped with Entellan (Merck, Germany) and examined with a Nikon Eclipse 55i (Nikon, Germany) microscope. Quantification of cell numbers was performed by manual counting the number of positive cells using Image J software. Cell numbers are expressed as cells/mm2.

Flow Cytometry

The complete corpus callosum was microdissected from PBS-perfused mice 14 days after progesterone pellet implantation. The tissue was placed into a Petri dish containing 2 ml of digestion buffer, 1 mg/ml of Collagenase D (Roche, Germany), 1 mg/ml of Neutral Protease (Worthington, UK), DNase I (Qiagen, UK), and diced into small pieces with a razor blade before incubation at 37°C for 30 min. Following the incubation, PBS was added to stop the enzymatic digestion and cells washed through a 70 µm filter with FACS buffer and centrifuged at 2,000 rpm for 5 min at 4°C. Isolated cells were incubated on ice for 5 min with anti-Olig2 antibody (1:100, Millipore, CA, USA) or anti-O4 antibody (1:50, Millipore, CA, USA). For the secondary antibody, goat anti-mouse antibody IgG fluorescein isothiocyanate (FITC) (1:100, Invitrogen, CA, USA) was used. Samples were analyzed with a FACSCalibur flow cytometer (BD Biosciences).

Statistics Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Data is reported as mean±SEM. $P<0.05$ were considered statistically significant.

Results

Light Microscope Examination

Histological examination of tissue sections was performed two weeks after treatment. Luxol fast blue histologic stain was used to assess the extent of remyelination in corpus callosum after implantation of progesterone pellet. High intensity staining was evident in the healthy control group (Figure 1A). Two weeks after progesterone implantation, an obvious remyelination was seen (Figure 1C), while less remyelination was seen in the placebo group (Figure 1B).

Quantification of myelin content in the body of corpus callosum (Figure 1D) shows that in progesterone pellet implanted mice there was a further significant increase in the remyelination score (1.65±0.1; $P\leq0.05$), compared with saline pellet implanted (placebo) mice (0.25±0.05; $P\leq0.05$).

Western Blot Assay

Densitometric measurements of immunoblots demonstrated that MBP and PLP contents were significantly lower in all treated groups compared with the healthy control group (Figure 2A). In the progesterone pellet implantation group, after two weeks of hormone administration, there were significant increase in MBP (0.72±0.1; $P\leq0.05$) contents compared with the placebo group (0.24±0.1; $P\leq0.05$) but less than the healthy control group (0.95±0.06; $P\leq0.05$) (Figure 2B). The PLP contents were significantly increased in progesterone receiving group (0.8±0.09; $P\leq0.05$) compared with the placebo group (0.1±0.03; $P\leq0.05$) but less than the healthy control group (0.99±0.08; $P\leq0.05$).

Progesterone Treatment Increased the Number of Oligodendrocyte and Oligodendroglial Progenitor Cells

As shown in Figure 3, progesterone treatment during 2 weeks after cuprizone-induced

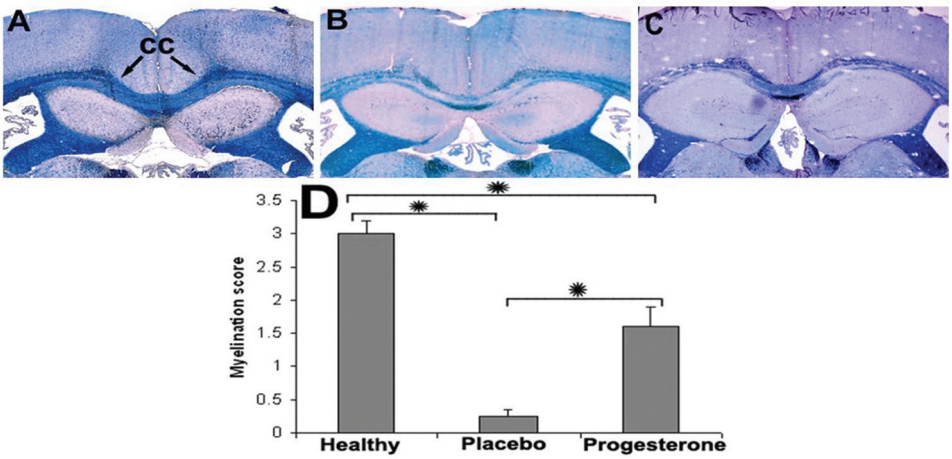

Figure 1: Remyelination in the corpus callosum two weeks after progesterone administration. Myelin content was evaluated by luxol fast blue staining. The photomicrographs were taken from sagital sections of body of corpus callosum two weeks after treatment. Corpus callosum (CC) of a healthy control group (A), placebo group (B), and progesterone implantation group (C). Quantification of myelin content in the body of corpus callosum (D) shows that in progesterone pellet implanted mice there was a further significant increase in the remyelination score compared with saline pellet implanted (placebo) mice. Data are represented as mean±SD (*P<0.05).

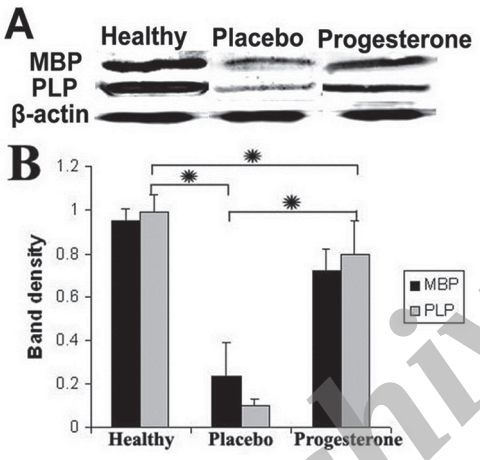

Figure 2: Protein expression of myelin basic protein (MBP) and proteolipid protein (PLP) were measured by Western blot and β actin was used as housekeeping control. A: The representative Western blot pictures of MBP and PLP protein in corpus callosum of the healthy control, placebo and progesterone administration mice. B: Bar chart showing the relative quantities of MBP and PLP measured densitometrically from the Western blots in different groups. The value represented here as mean±SEM of 3 mice in each group (*P<0.05).

demyelination, significantly increased the number of olig2+cells, an oligodendroglial progenitor cell marker, in body of corpus callosum of mice (Figures 3A, B, and C). The number of olig2+cells displayed a tendency to be higher in the placebo group (131±15; P≤0.05) when compared with the control health group (116±14; P≤0.05) without reaching statistical significant values (Figure 3D). In contrast, the number of olig2+cells was significantly higher in progesterone receiving group (327±17; P≤0.05) when compared with the placebo and control healthy groups (Figure 3D). In addition, olig2 and O4 expression was quantified by flow cytometry (Figure 4A). According to Figure 4C, the mean percentages of expression of olig2 decreased in the placebo group (4.27±1.2; P≤0.05) when compared with the control health group (5.54±0.64; P≤0.05) without reaching statistical significant values. Such decrease was significantly reversed in animals that received progesterone (40.06±1.4; P≤0.05).

Likewise, the mean percentages of expression of O4, an oligodendrocyte marker, was significantly increased in the progesterone group (24.4±0.6; P≤0.05) compared with the placebo group (1.52±0.2; P≤0.05), but less than the healthy control group (54.2±0.8; P≤0.05) (Figure 4B).

Discussion

Patients suffering from MS experience a significant decline in the rate of relapse during the third trimester of pregnancy and a significant increase during the first 3 months post-partum.[13] Thus, relapse decrease when levels of many hormones are elevated, and in particular those of progesterone, and increase when hormone levels decline to pre-pregnancy levels following delivery.[14]

Our study attempted to investigate the role of progesterone after experimentally induced demyelination in the male corpus callosum using cuprizone as toxic agent. To induce the demyelination, cuprizone model was used due to its reproducibility, simplicity, and low mortality.[15] Cuprizone feeding for several weeks

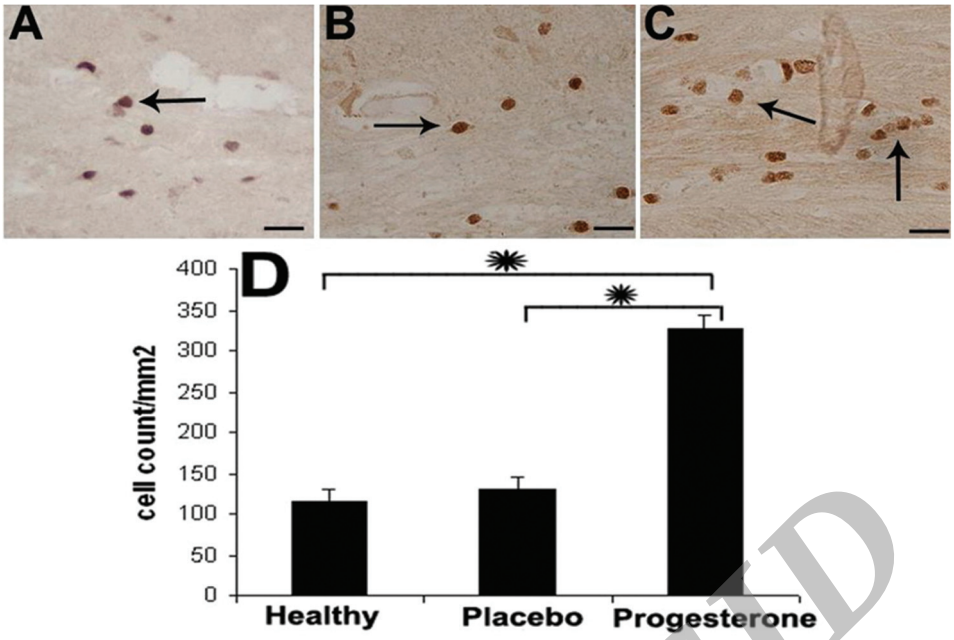

Figure 3: Effects of progesterone treatment on the number of olig2 positive cells (oligodendroglial progenitor cell) in the corpus callosum of cuprizone induced demyelination mice after two weeks of injury. Light photomicrographs of immunohistochemistry with anti-olig2 antibody (arrows) in healthy control group (A), placebo group (B), and progesterone implantation group (C). The cell counting of olig2 positive cells in body of the corpus callosum (D) shows that in progesterone pellet implanted mice there was a further significant increase in the number of olig2+ cells, compared with the healthy control and saline pellet implanted (placebo) mice. The value represented here as mean±SEM of 3 mice in each group (*P<0.05). Scale bars: 100 µ.

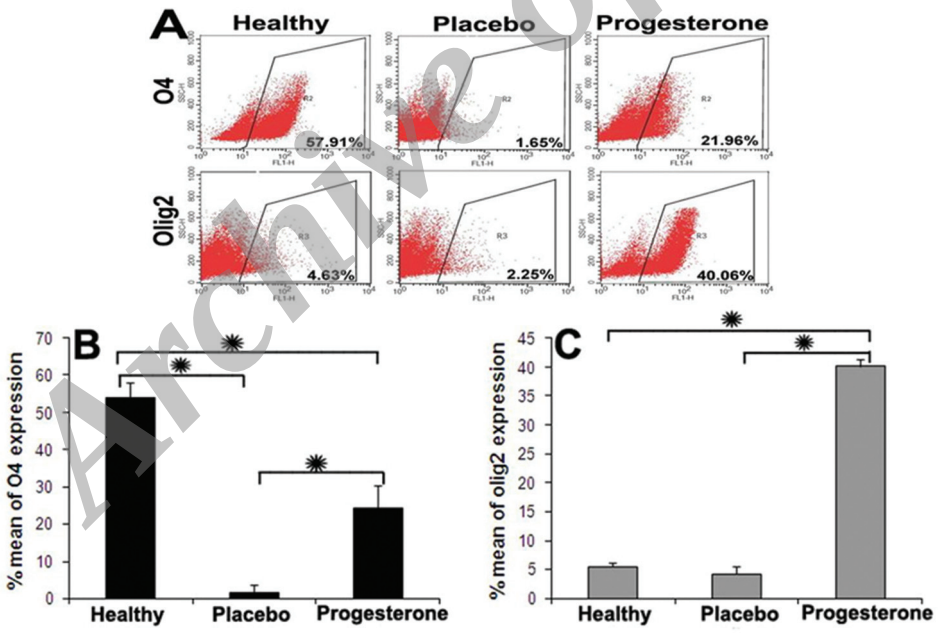

Figure 4: Oligodendrocyte and oligodendroglial progenitor cell numbers are markedly increased in the corpus callosum of progesterone receiving mice. Mononuclear cells were isolated from corpus callosum and the frequencies of Olig2 positive cells (oligodendroglial progenitor cell) and O4 positive cells (oligodendrocyte) determined using flow cytometry two weeks after treatment. Data shown in the Figure **A** are representative of the three performed independent experiments. The total number of O4 positive cells, shown in **B**, and the total number of Olig2 positive cells, shown in **C** of the healthy control, placebo, and progesterone-receiving mice are shown. The data are presented as the average of the cell counts from three mice per group ±SEM in **B** and **C** (*P<0.05).

causes massive demyelination of distinct white and grey matter brain region, including the corpus callosum.[16] Continued ingestion of the copper chelator cuprizone results in a white matter pathology that is similar to pattern III MS lesions.[17]

Our results show that progesterone increase remyelination in corpus callosum after a

demyelinating insult with cuprizone. A numerous experimental studies, have demonstrated the multiple actions of progesterone and its metabolites throughout the nervous system and their considerable influence on the functioning of the glial cells.[18] The influence of progesterone administration on the course of demyelination in experimental models for MS is well documented. For instance, some authors reported that administration of progesterone could attenuate demyelination in the lysolecithin-injured spinal cord.[3,10,19] Others have shown the application of progesterone possesses the capacity of inhibiting mature oligodendrocyte damage and reduced demyelination in cuprizone provoked demyelination of corpus callosum.[1,6] A particular asset of progesterone is that it not only could attenuate demyelination, but also promotes myelin formation either during development or during the remyelination of axons in the adult.[18]

Labombarda et al. demonstrated that short PROG treatment increased the number of OPC, increased the expression of MBP, and enhanced the expression of the Olig2 and Nkx2.2 transcription factors involved in specification and differentiation of the oligodendrocyte lineage.[19] In another study, mice were induced with EAE and treated with progesterone had a decreased clinical severity and enhanced expression of transcription factors essential for oligodendrocyte and myelin protein transcripts.[20] Hussain et al. showed that progesterone strongly increased the reappearance of cells of the oligodendroglial lineage in the demyelinated area.[9]

Although the cellular mechanisms responsible for the therapeutic effects of progesterone on remyelination remain unclear, however two hypotheses need to be considered. Progesterone as a neurosteroid hormone may participate to remyelination by either stimulating the proliferation and maturation of oligodendrocyte progenitor cells into mature oligodendrocytes able to form new myelin[1,3,6] or indirectly by modulating autoimmune and inflammatory processes.[21] However, in cuprizone-induced demyelination, an invasion of T or B cells is not observed.[1] Therefore, our study clearly shows that progesterone treatment is able to enhance remyelination of injured corpus callosum also independent of a B and T cell initiated autoimmune reaction.

In this study, results of immunohistochemistry and flow cytometry analysis show that progesterone treatment after cuprizone induced demyelination significantly increased the number of olig2+ cells (an oligodendroglial progenitor cell marker) and O4+ cells (an oligodendrocyte marker) in corpus callosum of mice. In addition, Western blot analysis shows that progesterone increased expression of MBP and PLP proteins. The remyelination of corpus callosum involves initially the recruitment of oligodendrocyte progenitors from the surrounding intact white matter into the area of demyelination and their subsequent differentiation into remyelinating oligodendrocytes.[3] The improvements of the remyelination rate that we find in progesterone treated animals may result from an enhancement in either or both of the recruitment and differentiation phases of remyelination. However, the signaling mechanisms involved in the myelinating actions of progesterone are not well defined. This is an important question since progesterone acts on multiple targets within the nervous system.[18] Although the molecular mechanisms employed by progesterone to improve remyelination after cuprizone demyelination induction were beyond the scope of our current study, some possibilities are worth mentioning. It has been shown that oligodendrocytes generation is a multiple-step process in which oligodendroglial progenitor cells proliferate, migrate, and differentiate into mature oligodendrocytes.[22] In a chemically induced multiple sclerosis model, remyelination is due to differentiation and maturation of adult oligodendroglial progenitor cells instead of pre-existing oligodendrocytes that suffer apoptotic cell death.[19] A stimulatory effect of progesterone on myelination was observed in cerebellar slices.[18] Progesterone was shown to stimulate the proliferation and maturation of oligodendrocyte progenitor cells in these slices. Recently, it was reported that progesterone increased the number of new oligodendrocytes[1,3,6] and this results supports the idea that the progesterone imposes a milieu favoring differentiation of proliferating progenitors into mature forms.[19]

Conclusion

The results obtained in the present study indicate that progesterone therapy enhanced remyelination of demyelinated corpus callosum axons in cuprizone model of MS.

Acknowledgment

This study was supported by Tehran University of Medical Sciences and Health Services, Tehran, Iran (grant No. 10926-30-02-89).

Conflict of interest: None declared.

References

1. Acs P, Kipp M, Norkute A, Johann S, Clarner T, Braun A, et al. 17beta-estradiol and

progesterone prevent cuprizone provoked demyelination of corpus callosum in male mice. Glia. 2009;57:807-14. doi: 10.1002/glia.20806. PubMed PMID: 19031445.
2. Crawford DK, Mangiardi M, Song B, Patel R, Du S, Sofroniew MV, et al. Oestrogen receptor beta ligand: a novel treatment to enhance endogenous functional remyelination. Brain. 2010;133:2999-3016. doi: 10.1093/brain/awq237. PubMed PMID: 20858739; PubMed Central PMCID: PMC2947430.
3. Ibanez C, Shields SA, El-Etr M, Baulieu EE, Schumacher M, Franklin RJ. Systemic progesterone administration results in a partial reversal of the age-associated decline in CNS remyelination following toxin-induced demyelination in male rats. Neuropathol Appl Neurobiol. 2004;30:80-9. doi: 10.1046/j.0305-1846.2003.00515.x. PubMed PMID: 14720179.
4. Kipp M, Beyer C. Impact of sex steroids on neuroinflammatory processes and experimental multiple sclerosis. Front Neuroendocrinol. 2009;30:188-200. doi: 10.1016/j.yfrne.2009.04.004. PubMed PMID: 19393685.
5. Houtchens MK. Pregnancy and multiple sclerosis. Semin Neurol. 2007;27:434-41. doi: 10.1055/s-2007-991127. PubMed PMID: 17940922.
6. Ye JN, Chen XS, Su L, Liu YL, Cai QY, Zhan XL, et al. Progesterone alleviates neural behavioral deficits and demyelination with reduced degeneration of oligodendroglial cells in cuprizone-induced mice. PLoS One. 2013;8:e54590. doi: 10.1371/journal.pone.0054590. PubMed PMID: 23359803; PubMed Central PMCID: PMC3554738.
7. Yu HJ, Fei J, Chen XS, Cai QY, Liu HL, Liu GD, et al. Progesterone attenuates neurological behavioral deficits of experimental autoimmune encephalomyelitis through remyelination with nucleus-sublocalized Olig1 protein. Neurosci Lett. 2010;476:42-5. doi: 10.1016/j.neulet.2010.03.079. PubMed PMID: 20381586.
8. Giatti S, Caruso D, Boraso M, Abbiati F, Ballarini E, Calabrese D, et al. Neuroprotective effects of progesterone in chronic experimental autoimmune encephalomyelitis. J Neuroendocrinol. 2012;24:851-61. doi: 10.1111/j.1365-2826.2012.02284.x. PubMed PMID: 22283602.
9. Hussain R, El-Etr M, Gaci O, Rakotomamonjy J, Macklin WB, Kumar N, et al. Progesterone and Nestorone facilitate axon remyelination: a role for progesterone receptors. Endocrinology. 2011;152:3820-31. doi: 10.1210/en.2011-1219. PubMed PMID: 21828184.
10. Garay L, Tungler V, Deniselle MC, Lima A, Roig P, De Nicola AF. Progesterone attenuates demyelination and microglial reaction in the lysolecithin-injured spinal cord. Neuroscience. 2011;192:588-97. doi: 10.1016/j.neuroscience.2011.06.065. PubMed PMID: 21736923.
11. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol. 2004;55:458-68. doi: 10.1002/ana.20016. PubMed PMID: 15048884.
12. Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol. 2001;11:107-16. doi: 10.1111/j.1750-3639.2001.tb00385.x. PubMed PMID: 11145196.
13. Gregg C, Shikar V, Larsen P, Mak G, Chojnacki A, Yong VW, et al. White matter plasticity and enhanced remyelination in the maternal CNS. J Neurosci. 2007;27:1812-23. doi: 10.1523/JNEUROSCI.4441-06.2007. PubMed PMID: 17314279.
14. Schumacher M, Guennoun R, Stein DG, De Nicola AF. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. Pharmacol Ther. 2007;116:77-106. doi: 10.1016/j.pharmthera.2007.06.001. PubMed PMID: 17659348.
15. Zendedel A, Beyer C, Kipp M. Cuprizone-induced demyelination as a tool to study remyelination and axonal protection. J Mol Neurosci. 2013;51:567-72. doi: 10.1007/s12031-013-0026-4. PubMed PMID: 23666824.
16. Clarner T, Diederichs F, Berger K, Denecke B, Gan L, van der Valk P, et al. Myelin debris regulates inflammatory responses in an experimental demyelination animal model and multiple sclerosis lesions. Glia. 2012;60:1468-80. doi: 10.1002/glia.22367. PubMed PMID: 22689449.
17. Steelman AJ, Thompson JP, Li J. Demyelination and remyelination in anatomically distinct regions of the corpus callosum following cuprizone intoxication. Neurosci Res. 2012;72:32-42. doi: 10.1016/j.neures.2011.10.002. PubMed PMID: 22015947; PubMed Central PMCID: PMC3230728.
18. Schumacher M, Guennoun R, Ghoumari A, Massaad C, Robert F, El-Etr M, et al. Novel perspectives for progesterone in hormone replacement therapy, with special reference

to the nervous system. Endocr Rev. 2007;28:387-439. doi: 10.1210/er.2006-0050. PubMed PMID: 17431228.
19. Labombarda F, Gonzalez SL, Lima A, Roig P, Guennoun R, Schumacher M, et al. Effects of progesterone on oligodendrocyte progenitors, oligodendrocyte transcription factors, and myelin proteins following spinal cord injury. Glia. 2009;57:884-97. doi: 10.1002/glia.20814. PubMed PMID: 19053058.
20. Garay LI, Gonzalez Deniselle MC, Brocca ME, Lima A, Roig P, De Nicola AF. Progesterone down-regulates spinal cord inflammatory mediators and increases myelination in experimental autoimmune encephalomyelitis. Neuroscience. 2012;226:40-50. doi: 10.1016/j.neuroscience.2012.09.032. PubMed PMID: 23000619.
21. El-Etr M, Ghoumari A, Sitruk-Ware R, Schumacher M. Hormonal influences in multiple sclerosis: new therapeutic benefits for steroids. Maturitas. 2011;68:47-51. doi: 10.1016/j.maturitas.2010.09.014. PubMed PMID: 21035281.
22. Baumann N, Pham-Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev. 2001;81:871-927. PubMed PMID: 11274346.