Original Article

Effects of Levothyroxine on Visual Evoked Potential Impairment Following Local Injections of Lysolecithin into the Rat Optic Chiasm

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Abstract

Background: Multiple sclerosis (MS) is a demyelinating disease of the central nervous system which has no any known definitive treatment. Studies have shown that thyroid hormones (THs) in addition to their roles in the development of the nervous system and the production of myelin have important roles in the adult's brain function. Since the only way to treat MS is the restoration of myelin, the aim of this study was to evaluate the effects of levothyroxine on visual evoked potential (VEP) impairment following local injections of lysolecithin into the rat optic chiasm. **Methods:** To induce demyelination, lysolecithin was injected into the optic chiasm of male Wistar rats. VEP recording was used to evaluate demyelination and remyelination before and 10, 17, and 24 days after the lysolecithin injection. The rats received an intraperitoneal injection of levothyroxine with doses 20, 50, and 100 μg/kg in different experimental groups. **Results:** VEP latency and amplitude showed demyelination at 10 and 17 days after an induced lesion in MS group which was reversed at day 24. Levothyroxine prevented these impairments, especially in high doses. **Conclusions:** According to the results, lysolecithin-induced demyelination at optic chiasm and VEP impairments can be restored by administration of levothyroxine. Therefore, THs probably have positive effects in demyelinating diseases.

Keywords: *Levothyroxine, lysolecithin, multiple sclerosis, optic chiasm, visual evoked potential*

Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system that primarily characterized by inflammation and demyelination. Furthermore, irreversible axonal damages have been observed at different stages of disease that considered as the main cause of disability and disease progression. So far, no effective treatment has been established to prevent the axonal and neuronal damages.[1] Visual system (optic nerve and/or optic chiasm) is impaired and demyelinated in about 70% of the people with MS, even before a definitive diagnosis of the disease.[2] Following the demyelinating attacks, especially in the visual system, some levels of remyelination occur that is depend on both the proliferation and migration of progenitor cells.[3] However, this remyelination is not sufficient and remains permanent lesions after each demyelination.[4] The only effective mechanism in the treatment of MS is the restoration of myelin that is performed by oligodendrocyte progenitor cells (OPC).[5] The proliferation and development of OPC to myelinating oligodendrocyte are affected by many factors including thyroid hormones (THs). One of the possible reasons for cessation of remyelination is a lack of adequate response of OPC to the stimulating factors.[6]

THs play an important role in the evolution of the nervous system from pre-birth to adulthood.[7] Recent studies showed that the TH plays an important role in the development of the nervous system in adults.[8] THs are essential for the development of oligodendrocytes as well as their migration into the demyelinated sites.[9] These hormones stimulate the construction of more oligodendrocytes from multipotential cells, and these actions occur not only during development but also in adulthood.[5]

Visual evoked potential (VEP) is an evoked electrophysiological response that can be extracted by signal averaging, from the electroencephalographic activity recorded from the scalp. VEP provides important information about the transmission of nerve signals in the visual system. VEP is often

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used as a noninvasive method of measuring visual function throughout the cortex.[2,10] The present study attempts to evaluate the effects of levothyroxine on remyelination of optic chiasm following lysolecithin-induced demyelination in adult rats by VEP.

Methods

Subjects

The subjects were male Wistar rats (250–300 g) that were housed five per cage and maintained on a 12 h light – dark cycle in an air conditioned constant temperature (23°C ± 1°C) room, with food and water made available *ad libitum*. The Ethic Committee for Animal Experiments at … University approved the study, and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Experimental groups were the sham, lesion (MS), MS-levothyroxine (MS-Th) 20 μg/kg, (MS-Th) 50 μg/kg, and (MS-Th) 100 μg/kg (n = 10).

The rats were anesthetized with chloral hydrates (400 mg/kg, intraperitoneal [IP])[11] and their heads were fixed in a stereotaxic frame. A heating pad was used to maintain body temperature at 36.5°C ± 0.5°C. For VEP recording, the skull was exposed, and two monopolar electrodes were implanted on the skull. The recording electrode was placed on the occipital part of the skull (AP: −7, L: 3) and the reference electrode was implanted on the anterior zone of the skull. The electrodes were connected to two small screws, which were fixed on the skull using dental cement.

After a VEP recording for baseline responses in the normal situation, a small hole was drilled on the skull and injection canula was lowered into the optic chiasm (anteroposterior = −0.3 mm; mediolateral = 0 mm; dorsoventral = −8.9 mm).[12] Injection canula was connected to a Hamilton syringe attached to a microinjector unit (Stoelting, USA). The lesion groups received an injection of 2 μl of 1% lysolecithin (Sigma, St. Louis, USA) prepared in sterile 0.9% saline, pH 7.4, into the chiasm.[13] The sham group underwent the same surgical procedures, but the same volume of saline was injected instead of lysolecithin. The solutions were injected within 3 min, and the needle was kept in place for another 5 min to avoid the possible reflux through the needle path. The animals were kept individually three days for recovery, and after that, the rats in different treated groups received IP injection of levothyroxine 20, 50, or 100 μg/kg (Sigma, St. Louis, USA)[14] for 21 days. Animals in the sham and the MS groups received the same volume of placebo. To assess the injection site coordination and the approximate area where lysolecithin might diffuse, Evan's Blue 2% was injected into the optic chiasm in accordance with stereotaxic coordinates.[15]

VEP is an evoked electrophysiological response that can be extracted by signal averaging, from the electroencephalographic activity. Animals were anesthetized with chloral hydrates (400 mg/kg, IP) and then fixed to stereotaxic apparatus. For VEP recording, animals were placed in a sound-isolated, dark and electrically shielded box. Stimulation included flashes of light (0.5 Hz for 300 s). Recorded responses were amplified (×10,000) and filtered (0.1 and 30 Hz band pass) (Electromodule D3111, Science Beam Institute, Tehran, Iran). Data were analyzed offline using eProbe software (Science Beam Institute, Tehran, Iran). For analyzing VEP, the latency between the flashlight and the first positive wave and the difference voltage between the peaks of first negative and first positive waves (P1-N1 peak); [Figure 1] were measured.[13] VEP was recorded before lysolecithin injection and 10, 17, and 24 days after that.

The results were analyzed using one-way analysis of variance followed by Tukey *post hoc*. The results are expressed as mean ± standard error of the mean. Furthermore, $P < 0.05$ was considered as the minimum statistically significant difference.

Results

Results showed that injection of lysolecithin in the optic chiasm significantly increased the latency of responses in the visual cortex of MS group with respect to the sham group after 10 and 17 days ($P < 0.001$ and $P < 0.05$, respectively); [Figure 2]. However, no significant differences were observed between the responses of MS and sham groups at day 24. Levothyroxine could not decrease the latency at dose 20 μg/kg, after 10 days and there was a significant difference between the sham and MS-Th 20 μg/kg ($P < 0.05$); [Figure 2]. Levothyroxine could bring the latency to the control levels in the MS groups at doses of 50 and 100 μg/kg during the 1st day and at dose of 20 μg/kg after 17 days [Figure 2].

The amplitude of P1–-N1 wave was significantly decreased in the MS group, 10 days after the injection of lysolecithin in optic chiasm ($P < 0.05$); [Figure 3]. However, no significant differences were observed between the responses of MS and sham groups at days 17 and 24. Levothyroxine increased the amplitude of a P1-N1 wave with all doses during all days. There is a significant difference between the MS and the MS-Tr 50 μg/kg groups 10 days postlesion ($P < 0.05$); [Figure 3].

Discussion

Our results, by evaluating the latency and amplitude of VEP, showed that on the tenth day and to a lesser extent on the seventeenth day after the injection of lysolecithin in optic chiasm of adult rats, a significant demyelination occurs. However, these changes reached to the levels of the control group after 24 days which represents remyelination. These results are consistent with previous studies that have shown maximum myelin damage occurs

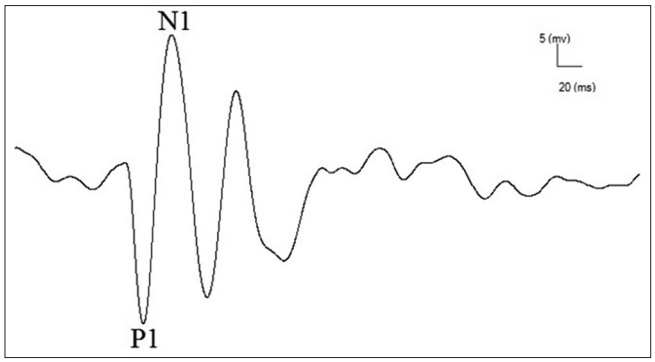

Figure 1: A graph of visual evoked potential recording. The latency was considered as the time between the flashlight and N1, and the amplitude was considered as the difference voltage between P1 and N1

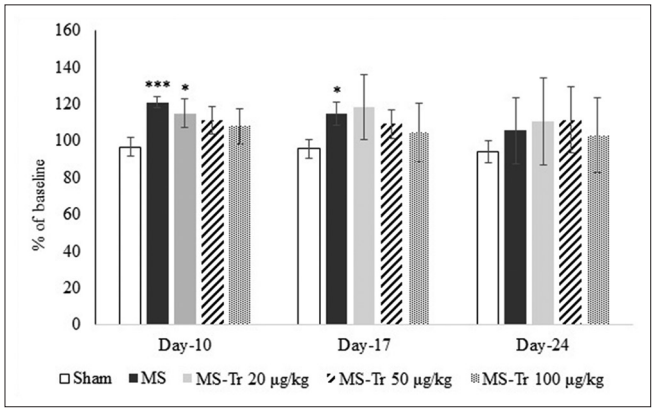

Figure 2: Effect of Levothyroxine on visual evoked potential after injection of lysolecithin in the optic chiasm of rats (multiple sclerosis is lesion group, and Tr is levothyroxine) at the latency between the flash light and the first positive wave. Values are shown as mean ± standard error of the mean. *$P < 0.05$ and ***$P < 0.001$ with respect to the sham group ($n = 10$)

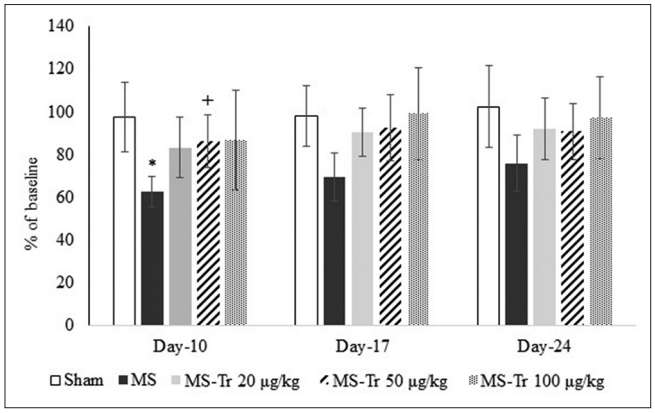

Figure 3: Effect of Levothyroxine on visual evoked potential after injection of lysolecithin in the optic chiasm of rats (multiple sclerosis is lesion group and Tr is levothyroxine) at the amplitude of P1-N1 peak. Values are shown as mean ± standard error of the mean. *$P < 0.05$ with respect to the sham group; +$P < 0.05$ with respect to the multiple sclerosis group ($n = 10$)

7–14 days after the injection of lysolecithin, and after which it is accompanied with remyelination.[16,17] Since both demyelination and remyelination occur in this model, therefore, it is an appropriate model for evaluating the therapeutic effects of different factors in different phases of MS. Optic chiasm is free of neuronal cell bodies; thus, it is a perfect place to assess the proliferation and differentiation of oligodendrocytes.[13] In this study, lysolecithin-induced demyelination and remyelination evaluated functionally using VEP recording. Lysolecithin causes local demyelination and is an appropriate model for evaluation of different phases of demyelination and remyelination.[15,18] VEP is also a good model for functional assessing of optic pathways and changes in VEPs amplitude and latency represent changes in the myelination level.[13]

THs play a critical role in brain organization and function at all stages of life.[8] Although these effects are well illustrated during prenatal development,[19] in adulthood also have dramatic effects on brain functions, the underlying mechanisms are less well known than the perinatal period.[8] Studies have shown that THs play an important role in promoting remyelination following injury or disease, and a deficiency of these hormones reduces the production of myelin.[9] In addition, the role of THs in adult neurogenesis is also shown.[8] In this study, from the fourth day after the injection of lysolecithin, the rats received levothyroxine for 21 days. According to the results on days 10 and 17, protective effects of levothyroxine were observed in the demyelination phase.

THs can be transmitted to the brain by transporters in the choroid plexus and affect all type of cells. Myelination is a TH-dependent process and studies have shown that THs play an important role in the production and development of oligodendrocytes.[5] Since remyelination needs to repeat the early developmental phases for myelination,[20] therefore, remyelination in MS is also dependent on THs. It has been demonstrated that in the acute phase of MS, proliferation of OPCs increases significantly, but they are not able to mature and produce myelinating oligodendrocytes.[21] This block of differentiation may be dependent to the cytokines including interferon-gamma that increase in the early stages of inflammation.[5] In addition, longer exposer of the progenitors to proinflammatory cytokines impairs their ability to produce OPCs and consequently OPC depilation in the chronic lesions.[22] Proinflammatory cytokines can also damage THs-derived OPC maturation. These cytokines through affecting the deiodinase and the expression of THs receptors cause localized hypothyroidism[23,24] and possibly deprive OPCs from stimulation of THs for differentiation to myelinating oligodendrocytes. Therefore, administration of THs in demyelinating diseases may be helpful, and our results confirm this claim.

One of the possible effects through which THs can affect the remyelination is affecting immune cells.[25] It has been demonstrated that administration of high-level thyroxine strongly suppressed T cell type cytokine transcription, significantly attenuated the proportions of Interferon-gamma (IFN-γ), IL-4 and IL-10-producing T lymphocytes and severely decreased the productions of

serum IFN-γ and IL-10.[26] Therefore, THs can prevent impaired OPC proliferation and differentiation to oligodendrocytes by reducing proinflammatory cytokines. In addition, THs can possibly affect demyelination and remyelination processes through affecting other cells, such as astrocytes,[27] as well as the construction and organization of cytoskeletal proteins[28] and extracellular matrix.[29]

In addition, in this study, we have observed that levothyroxine in doses of 50 and 100 μg/kg had the same effects on VEP and could prevent damages caused by the injection of lysolecithin. However, no alleviating effect was observed for low dose of levothyroxine (20 μg/kg) at the day 10. Thus, it can be suggested that high doses of THs that are associated with hyperthyroidism have more favorable effects on the healing process.

Conclusions

Our findings show that the administration of levothyroxine following the injection of lysolecithin to optic chiasm and resulting demyelination is effective in the restoring of myelin. The data correspond to the possibility that levothyroxine is helpful in remyelination in demyelinating diseases, especially with the concentrations that produce hyperthyroidism. Due to the serious complications of hyperthyroidism, advantageous of these beneficial effects on demyelination require further studies.

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Conflicts of interest

There are no conflicts of interest.

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