

Hypothyroidism Alleviates Cerebral Infarction but Exacerbates Blood-brain Barrier Disruption Following Transient Ischemic Stroke in Rats

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Abstract

Hypothyroidism is both a risk factor for stroke by disrupting fat metabolism and increasing atherosclerosis also a potential protective factor against its injuries. Hypothyroidism can influence the function of cerebral vessels such that BBB disruption is a reported complication of untreated hypothyroidism. The effects of hypothyroidism on cerebral vascular function in normal condition and following the ischemia-reperfusion is investigated in this study. Two groups of control euthyroid (CN) and hypothyroid (HPO) rats were studied. Hypothyroidism was induced by feeding methazole dissolved in drinking water (0.025 g/100 ml) for 4 weeks. After ensuring the hypothyroidism induction, cerebral ischemia-reperfusion was induced by MCAO. Following the reperfusion period, neuromotor dysfunction were evaluated and after removal of the brain, the volume of the infarct area was studied by the TTC staining and the BBB destruction was evaluated by the Evans Blue (EB) extravasation technique. Treatment with methimazole significantly reduced the levels of thyroid hormones (T3: 198 ± 4 vs. $73 \pm 3 \mu\text{g/dl}$ and T4: 3.08 ± 0.07 vs. $1.03 \pm 0.04 \mu\text{g/dl}$) in HPO. Furthermore, many clinical signs of hypothyroidism were developed in this group of animals. Hypothyroidism was associated with increased cerebral vascular permeability under non-ischemic conditions and exacerbation of BBB destruction after ischemia. However, neuromotor disorders and infarct volume were lower in hypothyroid animals than in euthyroid animals. Hypothyroidism has dual effects on ischemia-induced cerebrovascular damages. Aggravation of BBB destruction and alleviation of neuronal death following the cerebral ischemia-reperfusion are two converse effects of hypothyroidism. To clarify the exact mechanisms by which hypothyroidism causes these effects more investigations is required.

1. Introduction

High prevalence of stroke along with its high rate of mortality and disability (Seshadri, Beiser et al. 2006) raises the need for understanding the risk factors and also the factors exacerbating its damages. Given that hypothyroidism is associated with fat disorders and increased atherosclerosis, it is one of the putative risk factors for cardiovascular diseases (Biondi and Klein 2004). Although hypothyroidism is introduced as a potential risk factor for stroke (Qureshi, Suri et al. 2006, Karch and Thomas 2014), some clinical reports indicate that stroke occurrence in hypothyroid patients is associated with fewer damages and better outcome (Alevizaki, Synetou et al. 2006). Previous studies have provided different justifications for these dual and contrasting effects of hypothyroidism on stroke. Like its effect on other cardiovascular diseases, hypothyroidism, by disrupting the fat metabolism, increasing the risk of developing atherosclerosis and also hyperhomocysteinemia, might be a risk factor for stroke (Qureshi, Suri et al. 2006, Rimmel, Wanahita et al. 2006, Karch and Thomas 2014). On the other hand, the cerebral hypometabolic state, decreased sympathetic tone, and consequently more relaxed response to stress and preconditioning due to previous temporary ischemic attacks are the reasons for the protective role of hypothyroidism on stroke (Alevizaki, Synetou et al. 2006). Adverse effects of subclinical hypothyroidism (normal T4 level and high TSH level in plasma) on cardiovascular disease including stroke have also been clarified (Karakurum Goksel, Karatas et al. 2007, Giri, Edwards et al. 2014). However, fewer stroke-induced injuries and better functional outcome with the same reasons have been reported in subclinical hypothyroid patients (Baek, Chung et al. 2010, Akhoundi, Ghorbani et al. 2011).

Some animal studies have also shown that hypothyroid state, by reducing the release of excitatory neurotransmitters (Shuaib, Ijaz et al. 1994, Shuaib, Ijaz et al. 1994), reduction of oxidative stress and higher activity of antioxidant agents (Rastogi, Godbole et al. 2006), reduces the apoptosis and necrosis of the neurons and alleviates cerebral ischemia. On the other hand, it is reported that the protective effect of hypothyroidism on ischemic injuries is temporary (Lee, Yoo et al. 2010). Studying the hypothyroid gerbils for a longer time after induction of transient cerebral ischemia showed that hypothyroidism just delayed the neuronal death in the hippocampal CA1 region by temporarily reducing lipid peroxidation and increasing antioxidant activity, and over the time, it has had no effect on the extent of ischemia-induced neuronal death (Lee, Yoo et al. 2010).

Euthyroid sick syndrome (low total and free T3, normal T4 and TSH) which is a type of hormonal system response to severe stress is developed by various damages including stroke accompanies with decreased effects of active form of thyroid hormones on the body in critical conditions (Lee and Farwell 2016, Krysiak, Kedzia et al. 2017). Clinical studies have shown that there is a direct relationship between decreased T3 levels and the mortality rate or severity of stroke-induced damages (Alevizaki, Synetou et al. 2007, Dhital, Poudel et al. 2017). Contrary to the mentioned clinical reports, a recent animal study has shown that following ischemia, the activity of the cerebral D2 deiodinase enzyme increases, which results in more activity of thyroid hormones in the ischemic brain (Rastogi, Godbole et al. 2018). Using rT3 as an inhibitor of D2 enzyme could reduce the rate of ischemia-reperfusion damages in the studied animals (Rastogi, Godbole et al. 2018).

Disruption of BBB is one of the complications of cerebral ischemia. BBB disruption and associated brain edema will result in more pressure on the cerebral vessels, less penumbra blood supply, and more ischemia-related damages (Dirnagl, Iadecola et al. 1999). Some reports showed that untreated hypothyroidism accompanies with varying degrees of BBB damages evidenced by higher concentrations of macromolecules and cerebrospinal fluid proteins in hypothyroid animals or untreated hypothyroid patients (Thompson, Thompson et al. 1928, Schacht, Tourtellotte et al. 1968, Nystrom, Hamberger et al. 1997, Frost, Lee et al. 2004). Few researches have known dysfunction of the endothelial cells and glia cells as the reason for breaking BBB (Pancotto, Rossmesl et al. 2010). Heretofore, studies have not considered other hypothetical effects of hypothyroidism on cerebral ischemia because on one hand hypothyroidism can damage the normal function of the BBB and increase its permeability in non-ischemic condition and, on the other hand, it is associated with fewer post-stroke brain injuries. Therefore, the present study was designed to determine the function of the cerebral blood vessels in a normal state and following ischemia-reperfusion in hypothyroid animals and finally compare them with euthyroid animals.

2. Materials And Methods

Animals

The study was conducted on male adult Sprague–Dawley rats weighing 200–250 g obtained from the Animal House of Shiraz University of Medical Sciences. All procedures were in accordance with the Institutional Animal Ethics Committee of Shiraz University of Medical Sciences and followed the NIH Guidelines for

care and use of animals (NIH Publication No. 85–23, revised in 1996). The animals were kept under controlled temperature (22°C–24°C), humidity (40%–60%), and artificial 12-hour light/dark cycle, and were fed a regular diet (Pars Dam, Iran) and water *ad libitum*.

Experimental Protocol and Groups

Animals were randomly divided into one of two groups: hypothyroid (HPO) and control normal (CN). In the HPO group, the hypothyroid status was induced over a 4-week period by the oral administration of methimazole (Iran Hormone Company, Iran) in drinking water at a concentration of 0.025% w/v. In the CN group, the rats were treated in the same manner as the HPO group, except that they were given tap water for the 4-week period. At the end of the treatment period, the animals of each group were randomly divided for surgery as follows:

Ischemic CN (ICN, n=14) group: Surgery was accomplished at the neck region, and the right MCA was occluded for 60 minutes, followed by reopening and 24 hours of reperfusion.

Sham CN (SCN, n=8) group: All procedures were done like the ICN group, with the exception of MCA occlusion.

Ischemic HPO (IHPO, n=14) group: Experiments were performed like the ICN group.

Sham HPO (SHPO, n=8) group: All procedures were accomplished like the SCN group.

Subsequently, the rats of each group were further divided into 2 subgroups for the evaluation of: 1) cerebral infarct size and 2) BBB integrity. The overall success rate of the induction of brain ischemia was 60%, and the mortality rate of ischemia during the 24-hour period of reperfusion was 10% in all groups. The data presented in this study were obtained from animals that tolerated 60 minutes of brain ischemia and survived 24 hours after reperfusion.

Parameters Measured during the First Period of the Experiment

In both HPO and CN groups, the water intake was measured daily during the first week and every other day afterward. Body weight and systolic blood pressure (with a tail-cuff plethysmography and a Power Lab system) were measured once a week. The levels of thyroid hormones and core body temperature were checked at the beginning and the end of the treatment period. The blood sample was obtained by slightly anesthetizing the animal with isoflurane and collecting 1 mL of blood from the tip of the snipped tail. Afterwards, the samples were centrifuged (4,000 × g), and the separated serum was stored in a freezer (-70 °C) until further processing. Plasma T3 and T4 quantitative measurements were performed with ELISA (DiaPlus, Canada). In addition, the achieved hypothyroidism was confirmed by various clinical signs such as decreased body temperature, systolic blood pressure, bradycardia, and decreased body weight.

Surgical Procedures

The animals were fasting overnight prior to surgery, but they had free access to water. Anesthesia was induced through a facemask with 3% isoflurane in 30% oxygen and 70% nitrous oxide and maintained by 1.5% isoflurane in the same gas mixture. Continuous recording of blood pressure was done during surgery via cannulation of the right femoral artery. Similarly, core temperature was continuously recorded by means of rectal temperature probe and maintained at 37±0.5°C with a heating pad and a lamp.

Measurement of Regional Blood Flow

A laser Doppler flowmeter (AD Instrument, model: ML191, Australia) was used to measure the regional cerebral blood flow (rCBF) of the right hemisphere that receives its blood flow from the right MCA, according to the method described by Lecrux et al (Lecrux, Nicole et al. 2007). MCA occlusion was considered successful if there was a 75%–85% decrease in rCBF and a swift return to the pre-occluded level after reopening (Figure 1).

Induction of Focal Cerebral Ischemia

Transient focal cerebral ischemia was induced by using the intraluminal threaded technique of Longa et al. (Longa, Weinstein et al. 1989). Cerebral ischemia was started by advancing prepared silicon-coated nylon thread through the exposed right carotid artery up to the cranium and circle of Willis until the origin of MCA was occluded. Occlusion was confirmed by the observation of a sharp decline in rCBF trace. Reperfusion of the ischemic hemisphere, starting after pulling out the thread, was confirmed by restored rCBF. Finally, all instruments were removed, incisions were sutured, and the animals were allowed to recover from anesthesia and returned to a warm cage for recuperation during a 24-hour reperfusion period.

Evaluation of Neurological Disability

Evaluation of neurological motor function was carried out blindly on every animal 24 hours after surgery or ischemia by using a 5-point neurological deficit score (NDS) described previously. Then, the animals were sacrificed and their brain was prepared for the evaluation of ischemia infarct volume or BBB disruption.

Measurements of Cerebral Infarct Volume

Cerebral infarct volume was measured by 2,3,5-triphenyltetrazolium chloride staining method according to Swanson et al. (Swanson, Morton et al. 1990). After deep anesthesia with sodium thiopental, the animals were beheaded and their brains were removed, cleaned, and sliced. Staining was achieved with 2% TTC (2, 3, 5-triphenyltetrazolium chloride, Sigma), as previously described. The images of the slices were digitized by using a Cannon camera. The visible infarct zones were quantified using image analysis software. Finally, cerebral infarct volume was calculated, as described previously. Finally, cerebral infarct volume was calculated as described previously.

Quantitative Measurement of the Blood-Brain Barrier Permeability

The integrity of the brain vessels was quantitatively assessed using Evans Blue (EB) extravasation. After preparation, the optical density values of the prepared samples were measured at 620 nm with a Microplate reader (BioTek model: Gen 5, Germany). The tissue contents of EB were calculated according to the standard curves of dyes described, and the results are expressed as $\mu\text{g/g}$ wet tissue weight.

Statistical Analysis

All values are presented as means \pm SEMs. The analyses were performed using SPSS, version 21. ANOVA with repeated measures was used to evaluate the changes that had occurred during the 4-week treatment period in the body weight, daily drinking water, heart rate, blood pressure, and core temperature. One-way ANOVA with the Tukey post hoc test was used to evaluate variations in the rCBF, infarct volume, and BBB permeability. The Kruskal-Wallis test was also utilized to evaluate the NDS. Values of $P < 0.05$ were considered statistically significant.

3. Results

Circulating Levels of Thyroid Hormones, Systolic Blood Pressure, Heart Rate, Body Temperature, Water Consumption and Body Weight

Table 1 presents the plasma levels of T3 and T4 of the HPO group.

Table 1
Tail systolic blood pressure (SBP), heart rate (HR), water intake (water), body weight (BW), core temperature (Tem), and plasma concentration of T3 and T4 during treatment in control (CN) and hypothyroid (HPO) rats

Week	SBP (mmHg)				HR (beats/min)				Water (ml/day)				BW (g)				Tem(°C)		T ₃ ($\mu\text{g/dl}$)	
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	4	1	4
Group																				
CN	108 ± 2	110 ± 1	112 ± 2	116 ± 2	356 ± 3	360 ± 4	369 ± 4	374 ± 6	30 ± 0.6	31 ± 0.4	32 ± 1	30 ± 1	210 ± 3	225 ± 5	240 ± 1	257 ± 2	36.9 ± 0.06	37.1 ± 0.08	-	201 ± 6
HPO	111 ± 1	104 $\pm 2^*$	93 $\pm 1^*$	94 $\pm 2^*$	369 ± 4	332 $\pm 4^{**}$	305 $\pm 3^{**}$	299 $\pm 3^*$	32 ± 1	28 $\pm 1^*$	23 $\pm 1^*$	20 $\pm 1^{**}$	221 ± 2	228 ± 2	220 $\pm 1^*$	213 $\pm 1^*$	37 ± 0.05	36.1 $\pm 0.05^*$	196 ± 4	73 $\pm 3^{**}$

Data are presented as mean \pm SEM, *($p < 0.05$), ** ($p \leq 0.01$) significantly different from CN group.

Table 1 Tail systolic blood pressure (SBP), heart rate (HR), water intake (water), body weight (BW), core temperature (Tem), and plasma concentration of T3 and T4 during four weeks treatment in control (CN) and hypothyroid (HPO) rats.

The data presented in Table 1 show that the plasma levels of T3 and T4 of the HPO group were significantly lower than those in the CN group. This decrease indicated that treatment with methimazole had successfully induced experimental hypothyroidism in animals. Despite the sameness of systolic blood pressure, heart rate, and body temperature in both groups at the start of treatment, the systolic pressure, and heart rate of the HPO group decreased by 16% and 19% ($P < 0.01$) in the 4th week of methimazole treatment, respectively. Also, HPO animals experienced a notable decrease of core temperature after 4 weeks treatment with methimazole. While the daily water intake of the CN rats was unchanged during the treatment, water consumption in the HPO rats decreased significantly during the methimazole treatment reaching a minimum of 37.5% in the 4th week. In contrast to the steady increase in the body weight of the CN group, the HPO rats lost 3.6% of the body mass during the 4-week treatment period. The results of blood pressure recordings during the surgery are also presented in Table 2. These data indicated that the systolic blood pressure of the IHPO group was lower than that in the ICN group although this difference was not significant. There was no noticeable difference between the diastolic and mean pressure of both groups, and arterial pressure remained within normal limits pre-, during, and post-ischemia period in both groups. As was expected from the heart rate recorded prior to surgery, the heart rate in the IHPO group was significantly lower.

Table 2

The systolic, diastolic, mean arterial blood pressure (SBP, DBP, MBP) and heart rate (HR) before middle cerebral artery occlusion (MCA) occlusion, during occlusion (MCAO) and during reperfusion (Rep).

	SBP (mmHg)			DBP (mmHg)			MBP (mmHg)			HR (beats/min)		
	Before MCA	During MCAO	Rep	Before MCA	During MCAO	Rep	Before MCA	During MCAO	Rep	Before MCA	During MCAO	Rep
ICN (n = 14)	117 ± 3	121 ± 6	115 ± 5	83 ± 2	86 ± 3	84 ± 2	98 ± 5	102 ± 3	100 ± 4	372 ± 8	377 ± 10	383 ± 12
IHPO (n = 14)	105 ± 2	108 ± 2	107 ± 3	84 ± 1	87 ± 2	81 ± 2	93 ± 2	95 ± 2	92 ± 3	231 ± 13*	234 ± 10*	224 ± 11*
All values are mean ± SEM. Control ischemic (ICN); Ischemic hypothyroid (IHPO). *significantly different from ICN group ($p < 0.01$).												

Table 2 The systolic, diastolic, mean arterial blood pressure (SBP, DBP, MBP) and heart rate (HR) before middle cerebral artery occlusion (MCA) occlusion, during occlusion (MCAO) and during reperfusion (Rep)

Regional Cerebral Blood Flow

The rCBF before occlusion of MCA was considered as basal value and its alterations (% from baseline) with time are depicted in Fig. 1. There was no significant variation in rCBF in the sham groups of normal or HPO rats (SCN or SHPO). MCA occlusion was regarded to be successful when the rCBF fell to 75–85% of basal value in both ICN and IHPO groups, and there were no significant differences between them in this case. After MCA reopening, rCBF swiftly returned to its pre-occlusion level. Comparison of rCBF traces between the sham and ischemic groups indicated that hypothyroidism per se did not have any effect on the blood flow in the sham groups or during ischemia.

Figure 1 Regional cerebral blood flow (rCBF) in the sham control normal (SCN, n = 8), ischemic control normal (ICN, n = 4), sham hypothyroid (SHPO, n = 8), and ischemic hypothyroid (IHPO, n = 4) rats before and during middle cerebral artery occlusion (MCAO) and during the first 15-min reperfusion period. Values are presented as means ± SEMs

Neurological Disability Scales

The values of the NDS in the sham and ischemic rats are presented in Fig. 2. There was no neurological disability in the rats of the sham groups which showed anesthesia and neck surgery did not impair the neural activity. Compared with the NDS in the ICN group (2.15 ± 0.16), the score in the IHPO group (1.57 ± 0.11) was significantly reduced which denoted that the neural disability of ischemia was alleviated in the HPO rats.

Figure 2 Neurological deficit score (NDS) 24 hours after sham surgery or middle cerebral artery occlusion (MCAO) in the sham control (SCN), ischemic control (ICN), sham hypothyroid (SHPO), and ischemic hypothyroid (IHPO). All values are presented as means ± SEMs. *Significantly different from the SCN group or the SHPO group ($P = 0.01$). †Significantly different from the ICN group ($P = 0.05$)

Cerebral Infarct Volumes

MCA occlusion of 1 h followed by reperfusion for 24 h produced different magnitudes of infarctions in the right hemispheres of the ICN and IHPO rats without affecting the left sides (Fig. 3A). In comparison to the ICN group, a significant reduction in the volume of infarction was observed in the IHPO group, indicating that hypothyroidism attenuated the degree of infarction (129 ± 18 vs. 252 ± 16 mm³ Fig. 3B).

Figure 3a) Photographs illustrating the coronal sections of rat brain slices stained with triphenyltetrazolium chloride 24 h after 60 min MCAO in control ischemic (ICN) and hypothyroid ischemic (IHPO) rats. b) Infarct volumes of the right hemispheres of the ischemic control normal (ICN) and ischemic hypothyroid (IHPO) groups. All values are presented as means ± SEMs

Blood-Brain Barrier Integrity Disruption

In contrast to the absence of the blue color in both hemispheres of SCN or non-lesions of contralateral side of ICN, presence of EB in the brain of SHPO or non-ischemic hemisphere of IHPO revealed that hypothyroidism per se disrupted BBB integrity of this group of rats. In addition, compared to the ICN group, the concentration of EB dye in the right cerebral hemisphere of IHPO rats was markedly increased (32 ± 1 vs. 51 ± 5 µg/g), indicating that hypothyroidism significantly intensified ischemia BBB disruption in the HPO rats (Fig. 4).

Figure 4 Evans Blue (EB) concentrations (µg/g) in the left and the right hemispheres of the sham control (SCN), ischemic control (ICN), sham hypothyroid (SHPO), and ischemic hypothyroid (IHPO) rats. All values are presented as means ± SEMs

4. Discussion

The obtained results of this study showed two different effects of hypothyroidism on cerebral ischemia-reperfusion. Hypothyroidism on one hand reduced the infarct levels and neuromotor disorders, and on the other hand breakdown of BBB after cerebral ischemia was more severe in hypothyroid animals. The results of the current study showed that the induction of hypothyroidism for 4 weeks in rats was accompanied with some degrees of cerebrovascular injury and increased permeability of these vessels in non-ischemic conditions. Although hypothyroidism is considered as one of the risk factors for cardiovascular disease (Biondi and Klein 2004), the stroke damages in hypothyroid patients is milder with lower mortality and better outcome (Alevizaki, Synetou et al. 2006, Baek, Chung et al. 2010, Akhouni, Ghorbani et al. 2011, Oshinaike, Ogbera et al. 2015). These conflicting effects on stroke have also been reported in relation to several other risk factors for stroke including smoking (Bang, Park et al. 2007), hypertension (Yong, Diener et al. 2005), diabetes mellitus (Karapanayiotides, Piechowski-Jozwiak et al. 2004), and higher levels of serum cholesterol (Olsen, Christensen et al. 2007). Given that the mentioned conditions are known as risk factors for stroke, the results of some studies show that stroke in patients with these risk factors is associated with fewer injuries and better outcome. Studies on hypothyroid or subclinical hypothyroid patients suggest that low levels of brain metabolism as well as attenuation of response to stress due to reduced sensitivity to adrenergic stimuli in these patients lead to less stroke injuries and better recovery (Alevizaki, Synetou et al. 2006, Baek, Chung et al. 2010, Akhouni, Ghorbani et al. 2011). Bradycardia and lower blood pressure over 4 weeks of hypothyroidism induction in HPO group indicated the effect of low levels of thyroid hormones on the cardiovascular system and possibly decreased sensitivity of their adrenergic receptors. Also, decrease in body temperature, less weight gain in HPO animals, and lower water intake indicate lower body metabolism. Reducing the brain metabolism in a variety of ways, including lowering body temperature at the time of stroke is a way to reduce the cerebral injuries (Luan, Li et al. 2004). Therefore, one of the reasons for the lower neuromotor disorders and infarct level in HPO animals might be the decrease in adrenergic sensitivity and lower body metabolism. Although animal studies in this field are scarce and cannot provide accurate explanations for the effect of hypothyroidism on stroke, some factors have been investigated. Studies conducted in 1994 showed that the release of glutamate during ischemia in different parts of the brain of hypothyroid animals was lower than in euthyroid animals (Shuaib, Ijaz et al. 1994). Loss of ions balance in ischemic regions of the brain following the failure of energy-dependent transporters increases the glutamate release (Deb, Sharma et al. 2010). Activation of glutamate-dependent receptors and increased sodium and calcium entry into the cells will cause further damages including cell swelling, activation of apoptosis pathways and inflammation, increased oxidative stress factors, and peri-infarct depolarization (Deb, Sharma et al. 2010). Therefore, reduced glutamate release may attenuate other damaging factors in hypothyroid animals.

However, the reason for less glutamate release may be lower metabolism of hypothyroid animals and less dependence on oxygen and other nutrients. Another study found that lower oxidative stress factors and higher activity of antioxidant agents are the cause of less neuronal apoptosis and necrosis in ischemic hypothyroid animals during the reperfusion period of 24 hours (Rastogi, Godbole et al. 2006). However, another study has shown that these differences are temporary and with the prolongation of the reperfusion period, hypothyroidism cannot be effective in alleviating the ischemic brain injuries (Lee, Yoo et al. 2010). Extending the reperfusion period for 5 days caused no difference in neuronal death between hypothyroid and normal animals because the decrease in oxidative stress and increase in antioxidant factors in these animals were temporary; therefore, only delayed neuronal death occurred (Lee, Yoo et al. 2010). Since we evaluated the extent of injuries 24 hours after the ischemia; therefore, the results of this study are consistent with those of the studies with the same reperfusion period. Measuring the EB dye in the brain tissue 24h after reopening of MCA showed that BBB was more damaged in HPO animals than CN. Following the breakdown of the BBB and brain edema formation, compression of the cerebral vessels causes more reduction of penumbra perfusion and this intensifies the injuries (Dirnagl, Iadecola et al. 1999). Thus, it is possible that more severe damage to BBB in longer reperfusion periods may worsen the effects of cerebral ischemia in hypothyroid animals, and it may be a reason for the differences observed in the studies with different periods of reperfusion. Evaluation of the cerebral vessels in hypothyroid animals showed that the BBB of these animals in non-ischemic conditions were somewhat permeable to EB color and the rate of its damage after cerebral ischemia was more than that of the euthyroid animals. The result of studies on untreated hypothyroid patients showed dysfunction of the cerebral vessels in these patients as well as increased amount of proteins and other macromolecules in their cerebrospinal fluid (Thompson, Thompson et al. 1928, Schacht, Tourtellotte et al. 1968, Nystrom, Hamberger et al. 1997). Even, rare cases of papillae edema have been reported in hypothyroid patients (Frost, Lee et al. 2004). Alternative treatment with thyroid hormones ameliorates this defect (Thompson, Thompson et al. 1928, Schacht, Tourtellotte et al. 1968). However, studies have shown that reversible BBB disruption is influenced by factors such as duration and severity of the disease (Nystrom, Hamberger et al. 1997). Although the reason for damage to BBB of hypothyroid animals has not been investigated in this study, it has been shown that hypothyroidism accompanies with impaired function of the brain vascular endothelial cells and glial cells (Pancotto, Rossmesl et al. 2010), and lower density of cerebral vessels in hypothyroidism has also been reported (Schlenker, Hora et al. 2008). Induction of cerebral ischemia-reperfusion in hypothyroid animals worsens BBB disruption because the amount of EB which enters the brain tissue after stroke in these animals was higher than that in the euthyroid group. Whether further damage to the BBB can cause further brain injury in these animals over longer periods of reperfusion requires further investigation.

Measuring the animal body temperature during hypothyroidism induction showed that methimazole administration for 4 weeks reduced the body temperature to 1.1°C. This observed hypothermia, along with a decrease in weight gain and drinking rate indicates a decrease in the metabolism of hypothyroid animals. Results of some studies show that hypothermia can reduce the severity of stroke by lowering the level of metabolism. As in this study, we made efforts to make the same conditions at the time of surgery for all animals; the body temperature of the HPO animals was maintained at the time of surgery with the help of a heat lamp and a thermal pad in the temperature range of the CN animals. Furthermore, it was showed that hypothermia might have a protective role against ischemia if there is at least 2°C reduction in the body temperature (Luan, Li et al. 2004) or when temperature is less than 33°C (mild hypothermia) during induction of ischemia (Hayashi, Osuka et al. 2011). Because the maximal body temperature difference of the HPO and CN animals in this study was about 1.4°C, it is not possible to conclude that whether hypothermia at the time of reperfusion had any effect on brain injury or not. Whereas hypothyroidism has been shown to significantly decrease the blood flow and glucose metabolism in the brain (Constant, de Volder et al. 2001); therefore, the development of a hypo-metabolic state in the brain may be one of the causes of less injury to the brain of HPO animals 24 hours after one hour of middle cerebral artery occlusion. Investigation of the plasma levels of thyroid hormones in patients with stroke shows that there is a relationship between the severity of injury or mortality rate due to stroke and the plasma level of T3 (Chen, Wu et al. 2018, Lamba, Liu et al. 2018, Suda, Shimoyama et al. 2018). Decreased plasma levels

of thyroid hormones, especially T3 without apparent hypothyroidism (euthyroid sick syndrome), can occur in many critical situations including stroke (Lee and Farwell 2016, Krysiak, Kedzia et al. 2017). Euthyroid sick syndrome may result in reduced body metabolism in critical situations to reduce the severity of damage. The effectivity of alternative treatment with thyroid hormones in these critical conditions is suspicious (Maiden and Torpy 2019). Also, it is shown that treatment with thyroid hormones after stroke in the elderly mice is not effective in alleviating injuries (Akhoundzadeh, Vakili et al. 2019). In contrast with these findings, it has been shown that the activity of brain D2 enzyme is increased after ischemia-reperfusion (Margaill, Royer et al. 2005); this results in higher cerebral T3 level and spreading the neural damages (Rastogi, Godbole et al. 2018), so that the inhibition of D2 by rT3 results in reduced brain damage after ischemia-reperfusion (Rastogi, Godbole et al. 2018). Although hypothyroidism is not comparable with the acute decrease in thyroid hormones after the stroke, lowering thyroid hormones in any way can lessen the severity of the damages.

Overall, the findings of the present study suggest that hypothyroidism has dual effects on stroke in the studied animals. On one hand, it reduces the extent of infarction in the ischemic brain of animals and, on the other hand, exacerbates the cerebrovascular damages and enhances BBB disruption. It is possible that further damage to BBB may affect the size of the infarct area over time and extend it. Acceptance or rejection of this possibility requires further studies.

Declarations

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Conflicts of interest:

The authors declare that they have no conflict of interest.

Ethical approval:

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was approved by the local ethics committees by the number of IR.SUMS.REC. 1390. S6009

Availability of data and material:

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contribution:

All authors contributed to the study conception and design. Data collection and analysis as well as first drafting of the manuscript were performed by Mrs.Kehsvarz and the supervisor Mr.Dehghani revised the first manuscript. All authors read and approved the final manuscript.

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Figures

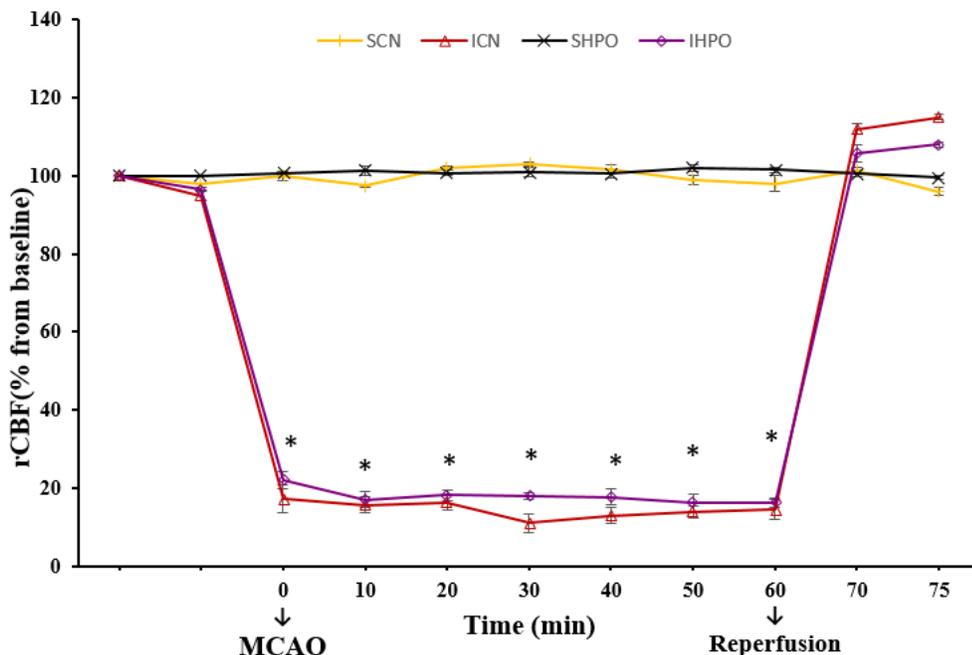


Figure 1

Regional cerebral blood flow (rCBF) in the sham control normal (SCN, n=8), ischemic control normal (ICN, n=4), sham hypothyroid (SHPO, n=8), and ischemic hypothyroid (IHPO, n=4) rats before and during middle cerebral artery occlusion (MCAO) and during the first 15-min reperfusion period. Values are presented as means±SEMs *Significantly different from the sham groups (P=0.0001).

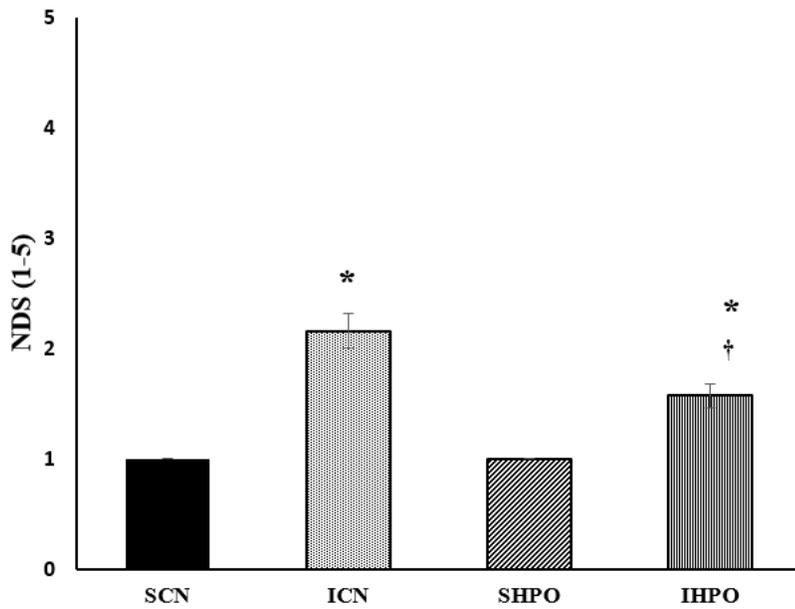


Figure 2
 Neurological deficit score (NDS) 24 hours after sham surgery or middle cerebral artery occlusion (MCAO) in the sham control (SCN), ischemic control (ICN), sham hypothyroid (SHPO), and ischemic hypothyroid (IHPO). All values are presented as means±SEMs. *Significantly different from the SCN group or the SHPO group (P=0.01). †Significantly different from the ICN group (P=0.05)

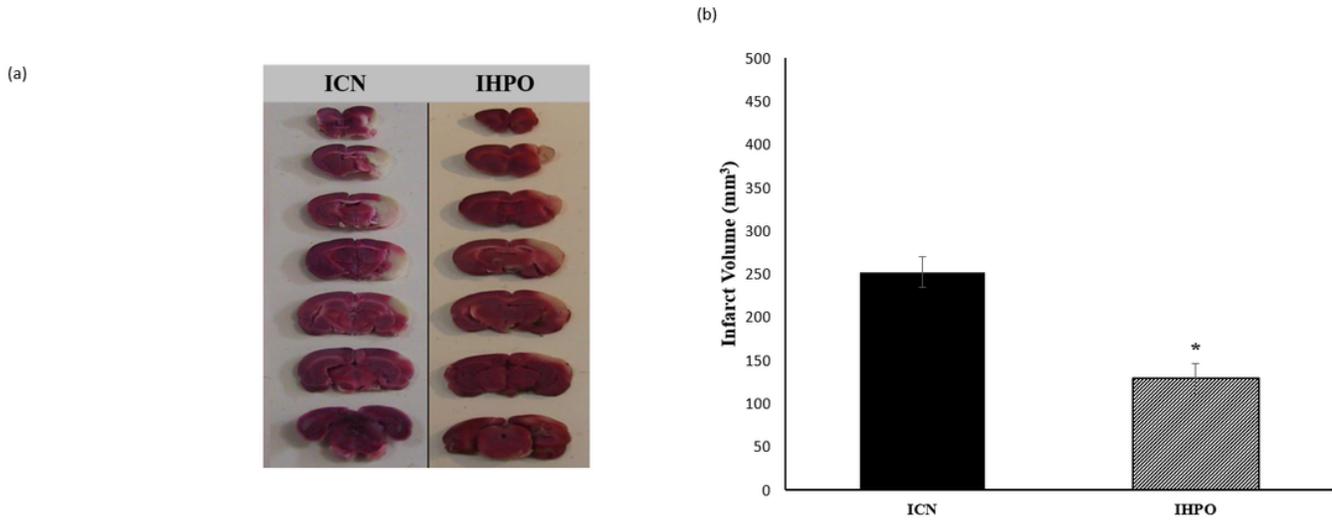


Figure 3
 a) Photographs illustrating the coronal sections of rat brain slices stained with triphenyltetrazolium chloride 24 h after 60 min MCAO in control ischemic (ICN) and hypothyroid ischemic (IHPO) rats. b) Infarct volumes of the right hemispheres of the ischemic control normal (ICN) and ischemic hypothyroid (IHPO) groups. All values are presented as means±SEMs *Significantly different from the ICN group (P=0.01).

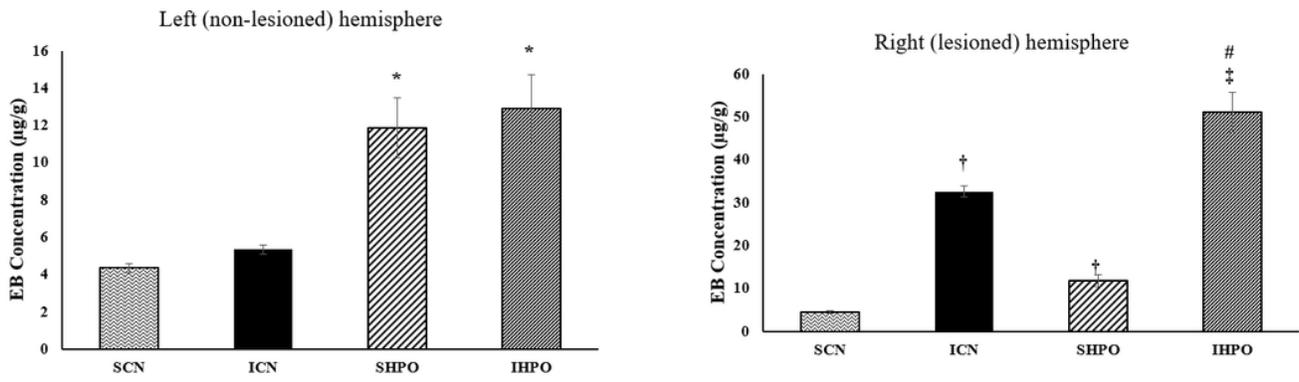


Figure 4

Evans Blue (EB) concentrations (µg/g) in the left and the right hemispheres of the sham control (SCN), ischemic control (ICN), sham hypothyroid (SHPO), and ischemic hypothyroid (IHPO) rats. All values are presented as means±SEMs * Values are significant from SCN or ICN, (P=0.01). # Significantly different from the ICN group (P=0.001) † Significantly different from the SCN group (P=0.001) ‡ Significantly different from the SHPO group (P=0.001)