Influence of Different Thyroid States on the Size of the Infarcted Zone, Neurological Disorder, MDA and LDH Following Middle Cerebral Artery Occlusion in Mice*

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Abstract Three different functional states of thyroid function were developed by medication with L-thyroxine (THY), dry thyroid tabulate, and tapazole (TAP) in mice. The responses to the middle cerebral artery occlusion were observed to assess their effects on the ischemic damage. The T3 and T4 in serum were increased in L-thyroxine and dry thyroid tabulate groups, and T3 was decreased but T4 was not changed in TAP medicated for 30 d. The infarcted mass, LDH & MDA were increased after MCAO in the L-thyroxine and dry thyroid tabulate groups, but decreased in TAP group. On the other hand, the neuronal deficiency was improved by L-thyroxin, vs the euthyroid, while there was no significant change in TAP group. It is concluded that hyperthyroidism exerts mainly a negative effect on cerebral infarction caused by MCAO.

Key words L-thyroxine; Tapazole; Middle cerebral artery occlusion; LDH; MDA

An infarcted zone could be developed after the cut off of oxygen supply by occlusion of the middle cerebral artery occlusion (MCAO) in mice. Oxygen demand of cerebral tissue is stimulated by L-thyroxine (THY) which enhances the oxidative-phosphorization in cells[1]. Thyroid hormone exerts its effect on many aspects in cardiovascular system[2]. An occlusion of blood supply would deteriorate the ischemic lesion in the presence of hyperthyroidism. An exaggerated cardiac infarcted zone by coronary occlusion was uncovered in rats with hyperthyroidism. The Na^+/K^+ ATPase activity, which is crucial for the function to maintain the normal membrane potential and the excitability of the cardiac and neural cells is depressed by an injury of ischemia. The function of THY is to cause enhancement of the sodium pump of the mitochondria of the heart and brain[3-6]. Therefore, it could be expected that THY is potential to provide a protection by an increment in Na^+/K^+ ATPase activity against the depressed Na^+/K^+ ATPase activity due to a shortage of oxygen supply. Neurological stress is a state causing an excess of endogenous bioactive substances including L-thyroxine and it may improve or benefit the cerebral neurons against ischemic insult. So it is interesting to get more insight on the likelihood of protection by the different thyroid functions on the cerebral infarcted zone by MCAO in mice.

1 Materials and Methods

Mice, 18 to 23 g of either sex, from the Animal House of the University, were used. Chemicals were offered as below; TTC from Shanghai General Factory of Distributing Chemical Reagents, L-thyroxine (THY) from Beijing Chemical Reagent Department, dry thyroid tabulate (DTT) from Shanghai Great Wall Pharmaceutical Factory, Tapazole (TAP) tabulate from Shanghai Yellow River Pharmaceutical Factory.

Three different thyroid states: Two hyperthyroidism states were developed in mice by po 1 mg/kg THY, or DTT 2 mg/kg suspended in 0.5% CMC for 10 days. Hypothyroidism was the result by

* Received Aug. 30, 1999

chronic oral medication of TAP 0.4 mg/kg suspended in 0.5% CMC for 30 days. Animals were killed 24 h after the last medication. The heart weight index (HWI) was calculated by heart weight(mg) / body weight (g)×100%.

Measurement of T3 and T4 in serum: Radio-immuno-assay[7] was applied to measure the serum T3 and T4 in blood samples which were taken from the post eyeball venous vessels on the day of experiment.

Middle cerebral artery occlusion [*MCAO*]: Mice were under light ether anesthesia and the skull was carefully operated on the left side to show the middle cerebral artery, then, it was occluded by heating using a heated stainless wire. The size of an infarcted zone, where was pale against the reddish survival tissue, was performed by TTC stain method.

MDA and LDH assay: These were done with kits purchased from the Nanjing Railway Medical College[8-10].

Evaluation of neurological deficits: Mice were evaluated carefully 24 h after occlusion for assessment of neurological status after occlusion of the MCA[11]. Spontaneous activity and deficiency in movement of the contralateral limb were monitored[12].

The statistic analysis: Data were expressed as means and standard deviation ($\bar{x} \pm s$). The student t test was applied to compute the statistic significance among the treated groups against the euthyroid group, at three levels as $P<0.05$, $P<0.05$, and $P<0.01$.

2 Results

2.1 *Influence on body and heart weight*

The changes in body and heart weight were shown in Tab 1. In the THY group the heart weight and the HWI were increased significantly against the normal. It was increased slightly but no statistic significance in DTT and no change was found in TAP groups versus THY (Tab 1).

Tab 1. Influence of L-thyroxine, dry thyroid and Tapazole on body and heart weight in mice. $\bar{x} \pm s$, $n=10$

Groups	Doses (mg/kg×10d)	BW (g)	HW (mg)	HWI (mg/g)
Normal	—	25.5±4.0	145±12	5.68±0.83
L-thyroxine	1	23.7±2.9	182±24[c]	7.68±1.3[c]
Dry thyroid	2	24.7±1.2	157±21[a,e]	6.3±61.3[a]
Tapazole	0.4mg/kg±30d	26.2±4.8	142±23[a,f]	5.42±1.6[a]

[a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$. vs normal. [e]$P<0.05$ [f]$P<0.01$ vs L-thyroxine.

2.2 *Changes in serum T3 and T4*

The values of serum T3 and T4 were greatly increased in THY group, with 16.4 fold in T3 and 28 fold in T4 compared with the euthyroid ($P<0.01$). There was a mild elevation of T3 and T4 found in DTT group, 2.24 fold in T3, and 16 fold in T4 vs TAP ($P<0.01$), but much less than THY ($P<0.01$). In contrast, T3 value was less in TAP group against the normal (Tab 2).

Tab 2. Changes in serum T3 and T4 by different thyroid states in mice. $\bar{x} \pm s$, $n=10$

Groups	Doses (mg/kg×10d)	T3 (ng/ml)	T4 (μg/ml)
Normal	—	0.59±0.17	0.6±0.3
L-thyroxine	1	9.71±5.11[c]	16.8±5.1[c]
Dry-thyroid	2	1.32±0.60[a,f]	9.6±5.9[a,f]
Tapazole	0.4mg/kg×30d	0.38±0.20[b]	0.6±0.3[b]

[b]$P<0.05$, [c]$P<0.01$ vs normal, [f]$P<0.01$, vs L-thyroxine.

2.3 *Influence on LDH activity*

As a result of cerebral ischemic damage, the level of LDH was increased significantly by MCAO in mice. An increment of 51% ($P<0.05$) in the model group over the normal LDH was seen after 24 h MCAO[data not shown] and the LDH values increased significantly in THY and DTT groups ($P<0.01$), but no change in TAP group was found as compared with the model (Tab 3).

2.4 *Influence on MDA production*

MDA production was enhanced as a consequence of ischemia by MCAO, and an increment was 187% more than the normal ($P<0.01$). There was no further increase seen in THY and DTTgroup, however, a reduction was found in

TAP group ($P<0.05$) (Tab 4).

Tab 3. Influence of different thyroid states on LDH activity in the affected cerebrum after MCAO in mice. $\bar{x}\pm s$, $n=10$

Groups	Dose(mg/kg×10d)	LDH(u/mg protein)
Model	—	0.49±0.07
L-thyroxine	1	0.63±0.12[b e]
Dry thyroid	2	0.62±0.05[a f]
Tapazole	0.4mg/kg×30d	0.44±0.10[a]

[a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ vs model, [e]$P<0.05$, [f]$P<0.01$ vs tapazole.

Tab 4. Influence on MDA production in different thyroid states in cerebrum after MCAO in mice. $\bar{x}\pm s$, $n=10$

Groups	Dose (mg/kg×10d)	MDA (nmol/mg protein)
Normal	—	0.20±0.03
Model	—	0.57±0.11[c]
L-thyroxine	1	0.60±0.08[c]
Dry thyroid	2	0.58±0.19[c]
Tapazole	0.4mg/kg×30d	0.48±0.13[a b]

[c]$P<0.01$ vs normal, [b]$P<0.05$ vs model.

2.5 *Influence on infarcted zone*

The infarcted zone was developed after 24 h after MCAO. The mass of infarcted zone in THY and DTT groups showed an increment of 33.3% & 26.9% respectively, versus the normal, however, a reduced size of 38.5% was found in TAP group (Tab 5).

Tab 5. Influence on infarcted zone by MCAO by different thyroid states in mice. $\bar{x}\pm s$, $n=10$

Groups	The affected hemisphere (mg)	Infarcted zone (mg)	Infarct mass %
Model	520±13	40.3±2.9	7.8±0.22
L-thyroxine	400±10.3	41.8±6.8[a]	10.4±0.66[c]
Dry thyroid	423±97	41.7±8.3[a]	9.9±0.86[c]
Tapazole	590±149	28.6±1.03[c]	4.8±0.7[c]

[a]$P>0.05$, [c]$P<0.01$ vs model.

2.6 *Effect on neurological deficits*

The values of spontaneous activity and deficiency in contra-limb movement were compared among the three groups. These were decreased in thyroxine, and dry thyroid groups, and no significant change was found in TAP groups (Tab 6).

Tab 6. Effect of L-thyroxine, dry thyroid and tapazole on neurological deficits by a scoring system after 24 h MCAO in mice. $\bar{x}\pm s$, $n=10$

Groups	Doses mg/kg	Spontaneous activity	Deficiency in contra-limb movement
Model	—	4.2±0.5	5.3±0.4
L-thyroxine	1	3.2±0.4[c]	4.2±0.9[c]
Dry thyroid	2	3.6±0.6[b]	4.5±1.0[b]
Tapazole	0.4mg/kg×30d	3.8±0.6[a]	4.7±1.2[a]

[a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ vs model.

3 Discussion

A hyperthyroid state was well established by THY, shown as an elevation in both the T3 &T4 and an increase in HWI. A less elevation in T3 and T4 in DTT group, is considered as a mild hyperthyroid state by THY, in agreement with the evidence of ventricular hypertrophy. Difference in thyroid states causes not only different responses to bioactive substances[13,14], but also the cerebral infarction mass. It was exacerbated in infarcted size and cardiac arrhythmias following coronary ligation[15].

The energy consumption was markedly increased as expected in THY group under an excess of T3 and T4 which stimulate catabolism and metabolism and a rapid heart rate and hypertrophied heart. There are two factors probably involved in developing cerebral ischemic lesion. One is oxygen demand which is enhanced by THY and another is the level of ATPase activity of the neurons. When cells are under anoxia by cutting off the blood supply, the Na/K ATPase activity will be reduced resultant with an impairment in membrane ion balance. It was found that the Na/K ATPase activity is increased by the stimulation of THY[16]. The increment in Na/K ATPase is favorable to the resistance to ischemic injury. So a state of hyperthyroidism does not exert only a negative effect to ischemic damage. The effect of TAP causing hypothyroidism[17,18] on the lesion developed by MCAO is also determined by the two factors fore-mentioned and showed a possible protec-

tion of cerebral neurons by a reduction in oxygen demand. So the influence of thyroid hormone, in the excess or decreased amount, may be bi-phasic in nature.

The hypertrophic myocardium induced by THY was evident. The effect of TAP was not evident on heart weight although T3 level in serum was reduced by TAP treatment.

The cerebral infarct mass developed after MCAO was exacerbated under the over-stimulation of THY, accompanied with an over-production of LDH and MDA in the affected cerebral region. These indicated that THY exerted an effect to make the ischemic events worse. The main outcome of an over-activity of THY caused an exaggerated consequence following an ischemic episode. However, a less neurological insult based on the changes in the motor regulating system and the spontaneous activity after MCAO in mice provided an improvement against the euthyroid state, suggesting that hyperthyroid state was impressed with a relief of neurological damage by an occlusion of the middle cerebral artery. A hypothyroid state produced by the treatment with TAP was effective to alleviate the mass of cerebral infarction, production of LDH and MDA release, but no beneficial result observed on the neurological derangement.

Although results of the investigation was conflicted to same extent, THY affects the ischemic lesion possibly in two directions, and the final outcome is the balance between the things developed on the two sides. It could be concluded that as a predominant factor an increase in oxygen consumption by THY is more crucial to impact a worse ischemic damage caused by MCAO and an elevated Na+/K+ ATPase activity by THY was not a determinant to blunt the ischemic lesion produced by MCAO.

Acknowledgement We thank Miss HE Yi and Miss CUI Yu-Jin for their kind assistance to manuscript and experimental procedures.

References

1 Dillmann WH. Biochemical basis of thyroid hormone action in the heart. *Am J Med*, 1990, **88**: 626

2 Ojamaak K, Samarel AM, Kupfer JM, *et al*. Thyroid hormone effects on cardiac gene expression independent of cardiac growth and protein syntheses. *Am J Physiol*, 1992, **263**: E534

3 Chen DD, Yu F, Dai Dezai. Reducing L-thyroxine cardiac hyperthrophy and superessing mitochondrial calcium pump by propranolol, verapamil and captopril. *J Pharm Univ*, 1995, **26**: 193

4 陈丁丁, 戴德哉. 维拉帕米和卡托普利对 L-甲状腺素诱发的大鼠心肌肥厚及左心室质膜 Na^+, K^+-ATP 酶活力升高的影响. 中国药理学与毒理学杂志, 1995, **9**: 265

5 陈丁丁, 于锋, 章涛等. 普萘洛尔, 维拉帕米和卡托普利对 L-甲状腺素性心肌肥厚的消退及抑制线粒体钙泵的作用. 中国药科大学学报, 1996, **27**: 44

6 陈丁丁, 戴德哉等. 普萘洛尔和苄普地尔消除左甲状腺素诱发的大鼠心肌肥厚和线粒体 $Ca^{2+}Mg^{2+}$-ATP 酶活力升高. 中国药理学报, 1996, **17**(6): 516

7 Ranjan D, Gunasekera & Hirosi Kuriyama. The influence of thyroid states upon responses of the rat aorta to catecholamines. *Br J Pharmacol*, 1990, **99**: 541

8 Promont C, Marie C, Bralet J. Increased Lipid Peroxidation in vulnerable brain regions after transient forebrain ischemia in rats. *Stroke*, 1989, **20**: 918

9 Hiroshi O, Nobuko O, Kunio Y. Assay for lipid peroxidas in animal tissues by thiobarbitric acid reaction. *Anal Bioch*, 1979, **95**: 351

10 Jae YK, Dennis WC. Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. *J Neurosci Methods*, 1987, **20**: 83

11 Zhi HH, Paul L, Huang NP, *et al*. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Sci*, 1994, **265**: 1683

12 Joshua BB, Lawrence HP, Miles T, *et al*. Rat middle cerebral artery occlusion. evaluation of the model and development of a neurologic examination. *Stroke*, 1986, **17**: 472

13 O'Donnell SR, Wanstall JC, Mustafa MBH. Influence of thyroid states on responses of rat isolated pulmonary artery vas difference and trachea to smooth muscle relexant drugs. *Br J Pharmacol*, 1987, **92**: 221

14 Stella RO, Donnell & Janet CW. Thyroxine treatment of aged or young rats demonstrates that vascular responses mediated by β-adrenoceptor subtypes can be differentially regulated. *Br J Pharmacol*, 1986, **88**: 41

15 于锋, 戴德哉. 左甲状腺素诱导的大鼠心肥厚加重心肌缺血损害及再灌心律失常. 中国药理学报, 1997, **18**(1): 71

16 Ralf P, Albert GB, Urs Ss, *et al*. The thyroid and the heart. *Circulation*, 1993, **87**: 1435

17 Poliker R, Kennedy B, Maisel A, *et al*. Decreased adrenergic sensitivity in patients with hypothyroidism. *J Am Coll Cardiol*, 1990, **15**: 94

18 Poliker R, Kennedy B, Ziegler M, *et al*. Decreased sensitivity to (-adrenergic stimulation in hypothyroid patients. *J Clin Endocrinol Metab*, 1990, **70**: 1761

不同甲状腺状态对小鼠大脑中动脉结扎所致梗塞区的大小及神经 MDA, LDH 的影响

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摘 要 目的: 观察给 L-甲状腺素及或给甲硫咪唑抑制甲状腺功能, 对实验性脑梗塞的影响。方法: 小鼠给药 L-甲状腺素、甲状腺干粉和甲硫咪唑造成三种不同的甲状腺功能水平。并观察他们对中脑动脉阻塞后反应来评价他们对缺血性损伤的作用。结果: 血清 T3 和 T4 在 L-甲状腺素组和甲状腺干粉组有所增加。甲硫咪唑组给药 30d 后, T3 值下降, T4 值不变。L-甲状腺素和甲状腺干粉组使梗塞区增加, LDH 活力和 MDA 增加。甲硫咪唑组梗塞范围下降。L-甲状腺素使神经病变减轻。小结: L-甲状腺素加重由 MCAO 所致的脑梗塞。

关键词 L-甲状腺素; 甲硫咪唑; 中脑动脉阻塞; LDH; MDA