Role of Thyroid Hormone in Modulating Astrocyte Activation

by

Payal Ghosh

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Co- Major Professor: Subhra Mohapatra, Ph. D.
Co- Major Professor: Daniel J Denmark, Ph. D.
Shyam S. Mohapatra, Ph. D.

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Dedication

To my Family

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Table of Contents

List of Figures

Abstract

Astrocytes are the specialized cells in the central nervous system (CNS). They play an important role in neuronal homeostasis. When activated, astrocytes may cause neuronal inflammation and degeneration. Thyroid hormones are important in maintaining neuronal development and homeostasis. Astrocytes play a pivotal role in this process. However, the role of levothyroxine (T4) in neuroinflammation is not clear. It is hypothesized that thyroid hormone treatment will reduce the inflammatory activation in astrocytes. Available oral T4 supplementations allow accumulation of T4 in the body and cause toxicity and side effects. It is hypothesized that the encapsulation of T4 in liposomes would reduce toxicity and possibly help in brain targeted delivery. Astrocytes were activated in vitro by bacterial lipopolysaccharide (LPS) treatment for 24 hours with or without T4. We observed that LPS treatment increased glial fibrillary acidic protein (GFAP) expression in the astrocytes and changed the morphology of the cells indicating their activation. Treatment with T4 hormone simultaneously or prophylactically significantly reduced the GFAP expression and changed the morphology of the cells similar to nonactivated ones. These observations indicate that the thyroid hormone has an anti-inflammatory effect on LPS- induced astrocytes activation. Moreover, a liposomal T4 formulation was synthesized and characterized. Preliminary studies suggest that liposomal T4 was safe and taken-up by astrocytes and reduced LPS-induced astrocyte activation. More studies are required to confirm these observations.

Chapter 1: Introduction

Inflammation and neuroinflammation

Inflammation is a pliable process to the harmful stimuli whether it is imparted by infection, trauma or necrotic tissue that a living organism is constantly exposed to(Varela, Mogildea, Moreno, & Lopes, 2018). Neuroinflammation can be defined as the inflammation of the nervous tissue, which can be initiated in response to a large variety of exogenous stimulations, infections, Traumatic Brain Injury, neurodegenerative disorders(Thibaut, 2017).Chronic neuroinflammation can induce neurodegeneration leading to neurodegenerative diseases, traumatic encephalopathy etc.(Sharp, Scott, & Leech, 2014). The neurodegenerative disorder causes the breakdown of nerve cells. Unlike other cells in the human system it is very difficult to recover the brain cells (Soloveva, Jamadar, Poudel, Georgiou-Karistianis, & Reviews, 2018).

Cells involved in neuroinflammation

Central Nervous System consists of different types of cells like oligodendrocytes, astrocytes, microglia, neurons. Neurons are the prime cells of the nervous system; oligodendrocyte produces the myelin sheath. Astrocyte provides the nutritional support to the neurons, microglia are the immune cells of the brain.

Astrocytes

Astrocytes are the star shaped glial cells in the central nervous system. They respond to different form of insults to the brain including injury, infection etc.by changing their morphology, proliferation and functional activity. At the time of inflammation or neuronal injury astrocytes

plays a pivotal role in maintaining the homeostatic balance by producing equilibrium of ions, fluid, water and blood flow (Burda, Bernstein, & Sofroniew, 2016). Astrocyte provides nutritional support to the neurons. They help to maintain the ionic balance in the extracellular fluid, secrete and uptake neuroactive factors and helps to maintain synaptic plasticity. Recent study shows that astrocyte sense and process neuronal signals through ion channels, neuro-transporters, and receptors on their membrane. (Zareh, Manshaei, Adibi, & Montazeri, 2019).

Astrocyte and neurodegeneration

Study shows that astrocyte expresses the membrane receptors for different neurotransmitters. Activated astrocyte has the ability to release a variety of neuroactive molecules including PGs, ATP (Fellin, Pascual, & Haydon, 2006).Astrocyte reciprocate to injury through their own functional reactions after becoming reactive astrocytes. They change their morphology or function depending on the severity of the damage(Acosta, Anderson, & Anderson, 2017). This phenomenon leads them to become either reactive astrocytes or protective astrocytes. The reactive astrocytes play an important role in neurodegeneration(Sung & Jimenez-Sanchez, 2020). Reactive astrocytes have been implicated in the pathogenesis of neurodegenerative diseases, for example, Alzheimer's disease, Parkinson's disease etc.(Seifert, Schilling, & Steinhäuser, 2006).

Microglia

Microglia are the immune cells of brain constituting 10-12% of the cells of central nervous system although the number depends upon the region. Following injury or infection, microglia rush to the site of injury or infection and phagocytose and mount immune response by releasing cytokines and chemokines.Microglia can induce protection by production of cytokines, and chemokines

which are proinflammatory mediator. Microglia also induces astrocyte activation and vice versa (Hickman, Izzy, Sen, Morsett, & El Khoury, 2018).

Neuroinflammation as a model of neurodegenerative Disease

Neurodegeneration, as the name suggests is the degradation of the neuronal cells. Neuroinflammation may cause irreversible damage to the neurons in the CNS. Unlike other organs like skin, liver, muscle, neuronal cells do not regenerate after damage by inflammation (Ransohoff, 2016). Thus, chronic neuroinflammation may lead to neurodegeneration. Lipo-polysaccharide (LPS) is a large molecule composed of lipid, polysaccharide and O-antigen and found in gram negative bacteria. To model neuroinflammation in laboratory settings LPS is widely used to treat cells. LPS activates the different cell types including astrocytes by inducing inflammatory signals. Human astrocytes are activated by administering 1ug/ml of LPS (Grabbe et al., 2020).

Neuroendocrine coordination

There is a sharp connection between neural, endocrine and immune system to maintain equilibrium in the human body. If this balance gets disturbed, then there cause some serious problems in the body.The brain gives signal to the glands to release the hormones.

Brain thyroid axis

The hypothalamus-pituitary-thyroid axis controls thyroid hormone production. The thyroid hormone releasing hormone(THRH) is released from the brain by and is controlled by GABAergic signals (Ref. needed). Itstimulates the anterior pituitary to releaseThyroid Stimulating Hormone (TSH) which send signals to the Thyroid glands to synthesize and secrete the thyroid hormone. Both the active forms of the thyroid hormone control the secretion of the TSH and TRH by

negative feedback mechanisms(Ortiga-Carvalho, Chiamolera, Pazos-Moura, & Wondisford, 2016).

Thyroid Hormone (TH)

Thyroid hormone is one of the important parts of endocrinal systems. Hypothalamus secretes Thyroid Stimulating Hormone (TSH) which acts on thyroid glands. Thyroid hormone secretes from thyroid glands which is anatomically situated in the anterior portion of the neck between C5 and T1 of the vertebrae. This is a butterfly shaped endocrinal gland divided into two lobes connected by the isthmus and are responsible for the production and secretion of thyroid hormone.

Thyroid hormoneaare amino acid derivatives made up of two tyrosine-based hormones named T_3 (triiodothyronine) and T_4 (thyroxine). T_4 contains 4 iodine atoms whereas T_3 contains 3 iodine atoms. T_3 or triiodothyronine is the most active form amongst them. Thyroid gland synthesizes thyroid hormone from the tyrosine and iodide (Fig 1).

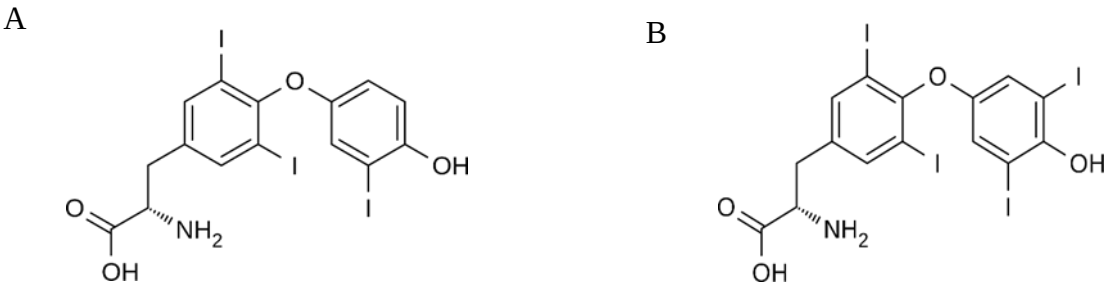


Figure 1. Molecular structures of Thyroid Hormone.

Being the major metabolic hormone in the body, thyroid hormones help to regulate fat, protein and carbohydrate metabolism and increases the body's sensitivity to adrenaline. It is the major

hormone regulating the neuronal development in pre and post-natal life. It also plays an important role in adult brain function (Schroederand Privalsky, 2014).

Brain development and Thyroid Hormone (TH)

Study shows that T_3 regulates the transcription of T_3 responsive genes. The action of thyroid hormone during brain development induces different aspects of neurogenesis. T_3 is crucial for the hippocampal development of the young adults (Krebs, 2017). Thyroid Hormone is one of the essential metabolic hormones which is responsible for proper development and expression of the cell in all vertebrate species. The deficiency in TH or delayed production can leads to slow growth of cells whereas excessive production can lead to hyperactivity and puffy eyes. It is found that proper function of gestational Thyroid hormone leads to healthy brain development and steady function of CNS. On the contrary impaired production in TH can lead to neurological/psychiatric disturbances. There are several symptoms like mood swings, apathy, atrophy, dementia, hypersomnia, anxiety. These symptoms increase with age and leads to various neurological/neurodegenerative disease. TH is needed for fetal cognitive development. At the time of gestation fetus are unable to develop thyroid hormone by its own. Maternal TH helps to do the same. Fetus brain starts forming in three weeks after pregnancy conception whereas rapid changes occurs in between 25^{th} to 40^{th} week of conception (Obregon, Escobar del Rey, & Morreale de Escobar, 2005). During this stage cognitive development of the fetus occurs in the embryo. Low production of thyroid hormone in pregnancy especially in midgestation period can lead to serious damage of central nervous system of both mother and fetus thus leads to impaired neurodevelopment. Maternal TH crosses placental barrier and bind with the deiodinases D2 and D3 receptors which is widely spread through the utero-placental epithelial tissue.

Thyroid Hormone and astrocytes

There are various ways to treat the astrocyte to recover from the process of neurodegeneration. An insufficient production of TH can lead to death of brain cells. A sufficient thyroid hormone production can nurture brain cells and prevent the degeneration of the cell. T_3 is the bioactive form but poorly available in the brain. Astrocytes converted T_4 to T_3 with the help of DiO2 enzyme. After conversion of T_3 it transferred to neurons for further conversion to T2(Krebs, 2017; Maher et al., 2016).

Hypothesis

Astrocyte acts as an avenue to transform the pro hormone (T_4) to its bioactive form (T_3) which can be transported to neurons. Activation of astrocyte is an important event initiating neuroinflammation. Treatment with T_4 is expected to inhibit the astrocyte activation and thereby neuroinflammation.

Specific Aims

- Development of a model of neurodegeneration
- Determination of the role of T_4 on activated astrocytes
- Development of a liposomal based method to deliver T_4 to the brain.

Chapter 2. Materials and Methods

Levothyroxine(T_4) preparation

Conjugation of T_4 and Cy5.5

T4 was purchased from Sigma-Aldrich. Stock solution (50 mg/ml) was made in 4 M methanolic ammonium Hydroxide.Cy5.5(Lumiprobe) conjugated T4 was prepared by using NHS-ester hydrolysis conjugation reaction.100 ug of T4 were dissolved in 0.1 M Na2CO3 and then the NHS ester was added to the solution and vortexed overnight. Then dialysis was done in dialysis bag (cut off size is 1000) to get rid of the unbounded dye or T4 for overnight in Nano-pure water.On the following dayT4 Cy5.5 conjugation was freeze dried in liophilizer overnight and then the resultant powder kept in 4'C.Preparation of Liposome particles

Preparation of liposome

The liposome was prepared using thin film hydrating method (). A solution of 100 uL of Cholesterol (5 mg/mL), 372 uL of DSPE (5 mg/mL) and 378 uL of DPPC (5 mg/mL) was prepared in Chloroform. All lipids together were added in a round bottom flask. The flask was connected to the rotary evaporator initially heated at 40 'C. The flask was inclined in such a way that the solution of the lipid covered most of the surface of flask and formed the uniform film. The flask was rotated at 100 rpm until the solution forms a uniform film in the bottom of the flask. Then the flask was

covered with para-film and placed in vacuum dryer overnight for solvent residual solvent evaporation.

Encapsulation of T_4-Cy5.5 into liposome:

1ml T_4-Cy5.5 solution which was dissolved in 4M Ammonium Hydroxide in water added in the round bottom flask. Then the flask was again connected to the rotary evaporator with heating at 40'C and the flask was rotated at 100 rpm. After 15 minutes, the heat was lower down at the 20'C. After 1hr, liposome encapsulated particles were collected. Then the particles were bathsonicated for 15 minutes. Then the particles were filtered 0.2 um syringe filter to ensure the separation of large particles from smaller particles.

Characterization of liposome encapsulated T4-Cy5.5

Particle Size and charge determination by DLS and zetasizer. The particle size distribution of liposome encapsulated T_4-Cy5.5 particles was determined using dynamic light scattering (DLS) technique. The particle size distribution and Zeta potential were measured using a Malvern Zetasizer Nano ZS (Malvern instruments, Worcestershire, UK). The Liposome encapsulated T_4-Cy5.5 solution was diluted to 1:10 with deionized water and placed in disposable folded capillary cell cuvette and the scattering intensity was measured in triplicate for each sample at 25'C.

In vitro study

Human astrocyte cell culture

Human astrocyte cells were purchased from Life technologies. Astrocyte cells were propagated using Endothelial Growth Media (EGM) supplemented with 2% FBS (Sigma-Aldrich), and growth

factor (Thermo Fischer), 1% Penicillin-Streptomycin () to limit contaminations. Cells were passaged every 4 days and maintained at 37' C and 5 % CO2.

Activation of astrocytes with LPS

On 80 -90% confluency, cells were detached by trypsinization and plated in a 96 well plate at a density of 5000 cells per well. Following day after plating, cells were washed with serum free media and treated with 1 ug/ml LPS in serum depleted media for 24 hours.

Treatment of astrocytes with T4

Cells were detached by trypsinization and plated in a 96 well plate at a density of 5K per well. On following day cells were washed with serum free media and treated with 50nM, 25nM, and 12.5nM T_4 in serum depleted media for 24hrs to observe the cell viability. Also, these dosages were used along with LPS, followed by LPS treatment and also pre-LPS treatment for 24hrs.

Cell uptake of Cy5.5,T_4-Cy5.5, Liposome- T_4-Cy5.5

In a 96 well plate, at a density of 5000 cells per well seeded to get initial 70-75% confluency for the experiment on the following day. Three such plates were prepared for each time point. After 24hrs after plating cells were washed with serum free media and treated with Cy5.5, T_4-Cy5.5, Liposome- T_4-Cy5.5 at different time points (1hr and 3hrs) and incubated at 37'C in a humidified incubator with 5% CO2. After each time point the cells were fixed 4% PFA for 30 minutes and washed thrice with PBS to remove excess amount of PFA. Then 100uL of PBS with DAPI (1:1000) was added to each well and incubated at room temperature for 15 minutes. Cells were washed with PBS and observed under the Olympus microscope.

Statistical Analysis

Data are expressed as mean ± SEM. Student's t-test was used to compare between means.

A p value less than 0.05 is considered as significant.

Chapter 3. Results and Discussion

Activation of astrocytes by LPS

Cell viability

Treatment of astrocytes with 0.5ug/ml LPS did not cause any cell loss. Significant cell loss was observed when astrocytes were treated with 0.5 ug/ml for 48 h or with 1 ug/ml LPS for either 24 hours or 48 hours.

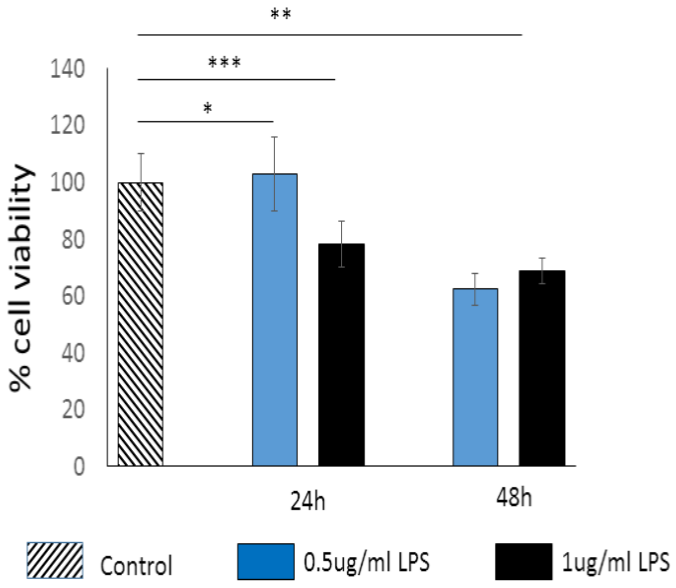


Figure 2. . Cell viability of activated astrocytes

Effect on GFAP expression

Astrocytes express basal level of GFAP. Following activation by 0.5ug/ml LPS for 24 hours, GFAP expression did not change significantly while 48h treatment increased GFAP significantly (Fig 2A, upper panel 0.5 ug/ml treatment for 24h and 48h; bottom panel 1 ug/ml treatment for 24h and 48h; 2B. histogram of GFAP intensity per cell)

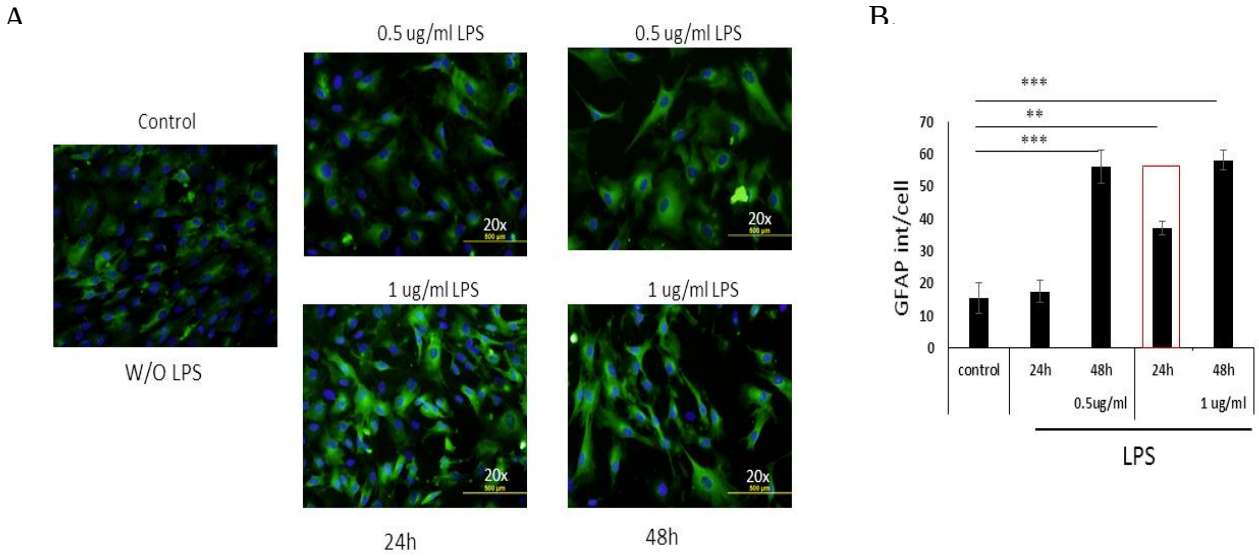


Figure 3. Astrocytes are activated by LPS

Astrocytes are activated by LPS. A, Representative immunofluorescence images showing GFAP expression in astrocytes with or without LPS treatment. B, histograms showing imageJ quantitation of GFAP expression in astrocytes. Scale bar 200u, **p<0.01, ***p<0.001.

Determination of optimum dose of T4 for treatment of LPS-activated astrocytes

Uptake of T_4-cy5.5 by astrocytes

Astrocytes were cultured in the presence of LPS and Cy5.5 for 1h or 3h and cy5.5 uptake examined by fluorescent microscopy. Cy5.5 uptake is shown in Fig 3C, upper panel naïve and control at 3 hours; middle panel free Cy5.5 uptake at 1 hour and 3 hours; down panel T4-Cy5.5 uptake at 1 hour and 3 hours; 3D. histogram of Cy5.5 intensity per cell.

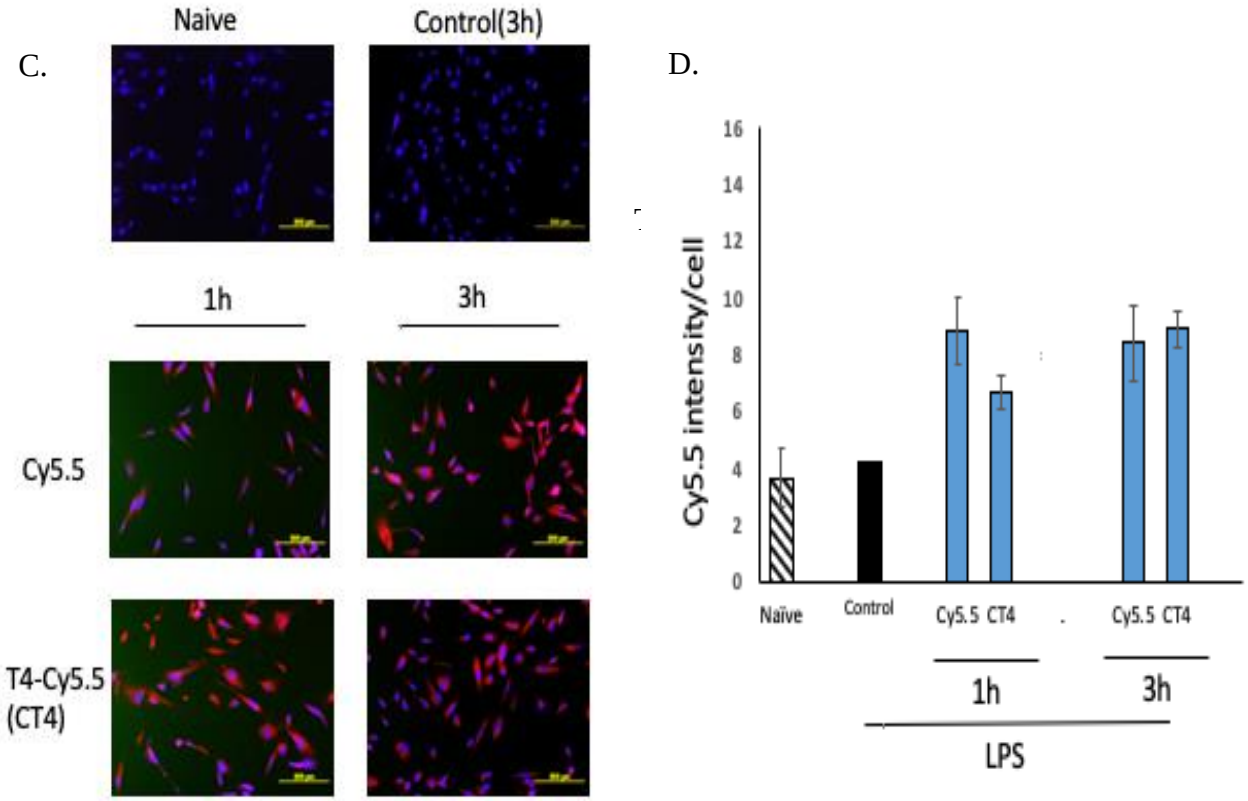


Figure 4. Cy5.5 uptake in LPS activated astrocytes.

Cy5.5 uptake in LPS activated astrocytes. C, Representative immunofluorescence images showing Cy5.5 uptake in astrocytes with LPS treatment. D, histograms showing imageJ quantitation of GFAP expression in astrocytes. Scale bar 200u, **p<0.01, ***p<0.001.

We examined effects of T4 on astrocyte cell viability. Astrocytes were cultured in the presence of varying concentrations of T4 and cell viability measured by CTG assay. Treatment with 50 nM of T4 for 24 h caused sa significant cell loss. No change in viability noticed when astrocytes were treated with 12.5 or 25 nM T4 (Figure 4). Hence, 25 nM T4 was chosen for subsequent experiment where **p<0.01, ***p<0.001.

Effect of T4 treatment on viability of activated astrocytes:

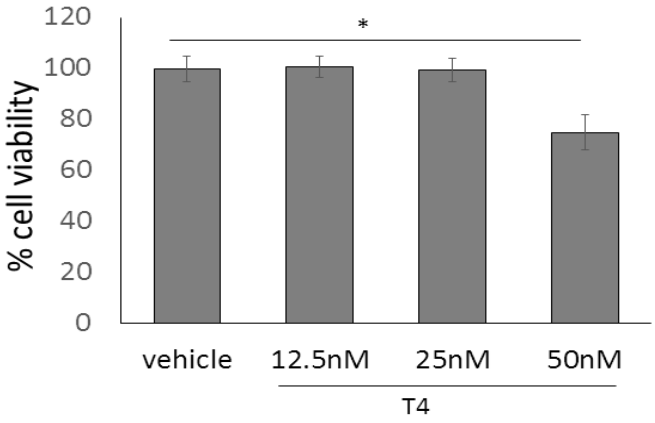


Figure 5. % Cell viability of astrocytes in presence of different doses of T4.

Astrocytes were stimulated by LPS in the presence of varying doses of T4 and astrocyte cell viability examined by CTG assay. We found that T4 (50 nM,25 nM,12.5 nM) administered before LPS activation did not alter LPS induced loss of cell viability whereas T4 treatment simultaneously with LPS or followed by LPS activation improved astrocyte viability. 25 nM T4 dose shows significance. Improvement of cell viability when administered simultaneously.

Effect of T4 treatment on GFAP expression of activated astrocytes

In this experiment, we examined the effects of T4 treatment on GFAP expression. Following activation of astrocytes by 0.5ug/ml LPS for 24 hours GFAP expression did not change significantly while 48h treatment increased GFAP significantly (Fig 6E, upper panel: naïve, LPS(1ug/ml); second panel: LPS(1ug/ml) and T_4 (50 nM, 25 nM, 12.5 nM) treatment together;

third panel: T_4 (50 nM, 25 nM, 12.5 nM) treatment followed by LPS(1ug/ml) activation; fourth panel: T_4 (50 nM, 25 nM, 12.5 nM) treatment followed by LPS treatment.)

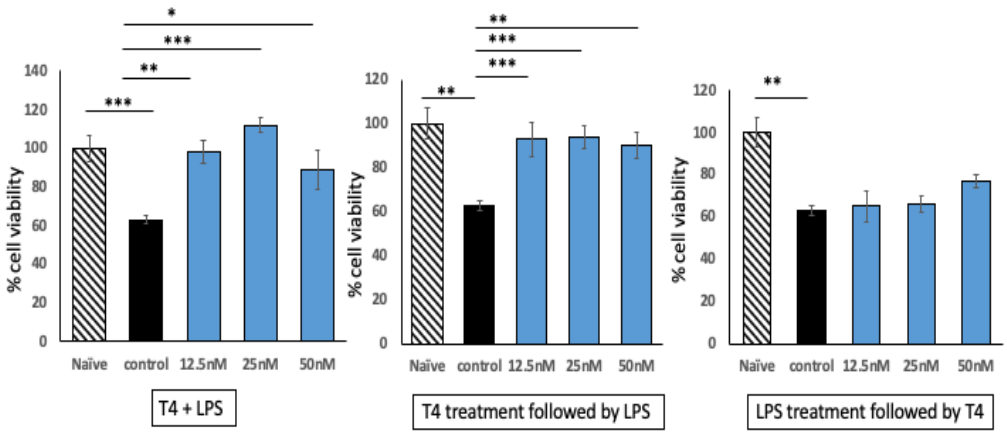


Figure 6 Cell viability of activated astrocytes when treated with T4 simultaneously followed by LPS or before LPS treatment. Representative histograms showing % cell viability of astrocytes. **p<0.01, ***p<0.001.

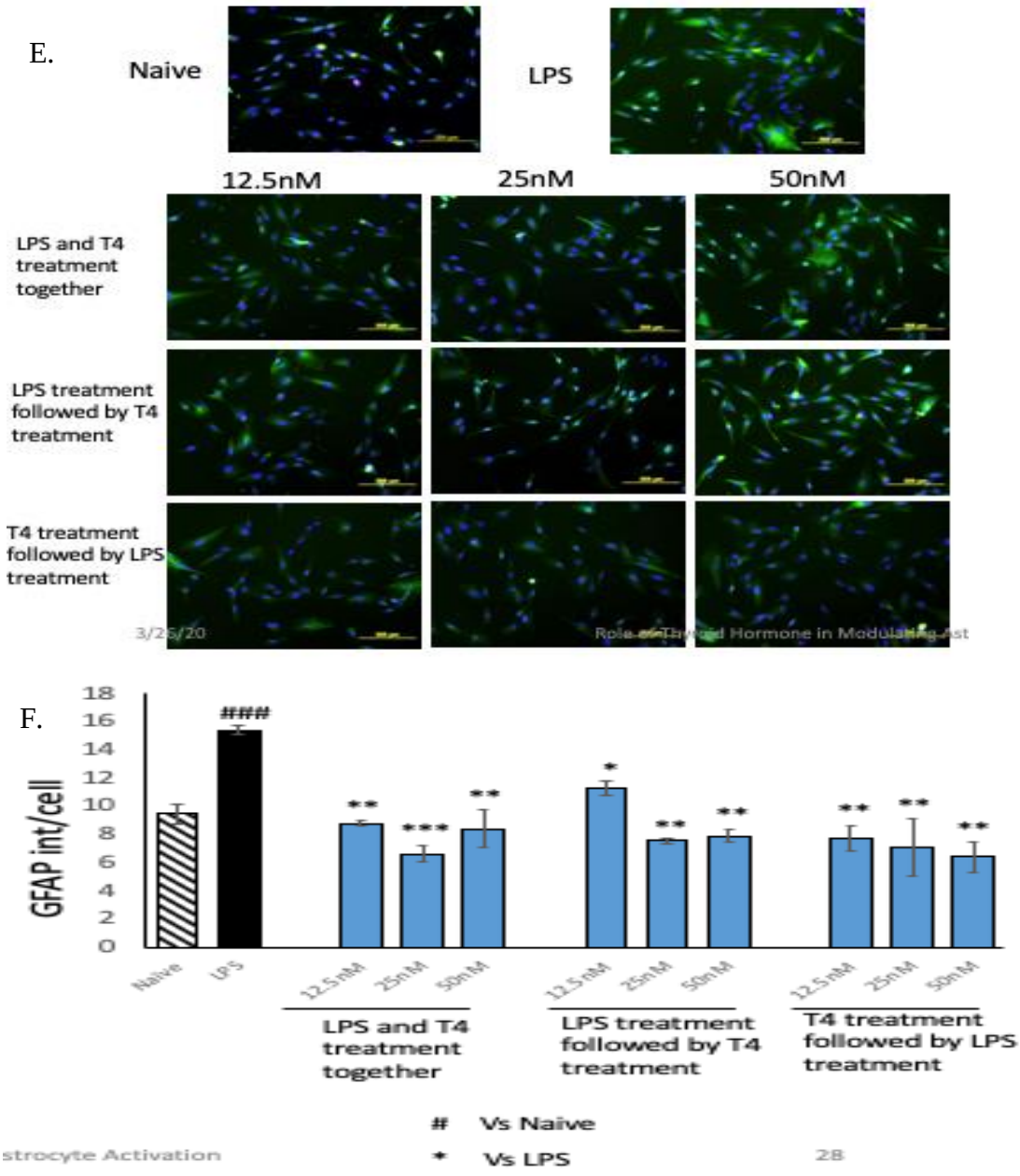


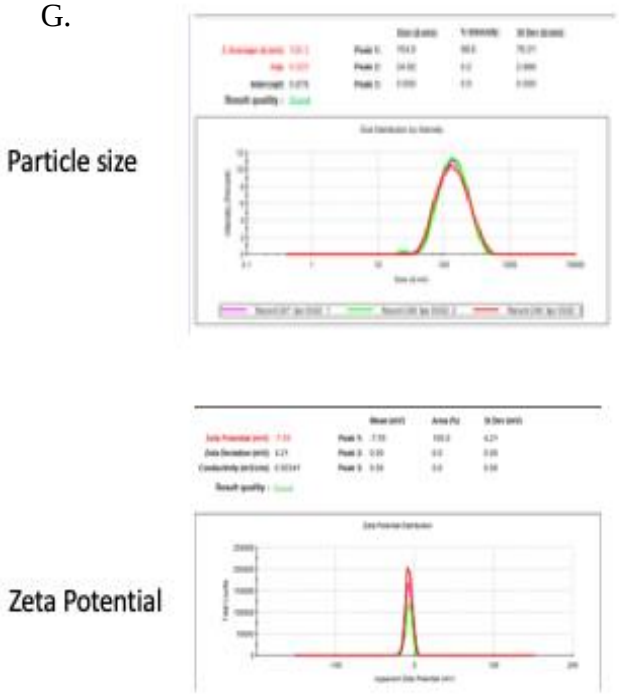
Figure 7. immunofluorescence images showing GFAP expression in astrocytes when T4 treatment is done

Characterization of T4-liposome nanoparticle

Particle Size and Zeta Potential of the nanoparticle

Figure 8 shows the particle size and zeta potential of liposomal encapsulated T4

G.



H.

	Particle Size	Zeta Potential
	110	-7.7
	126	-10.6
	220	-6.92
avg	152	-8.40
±SD	59.43	1.93

Figure 8. G. The particle size and zeta potential of the liposome-T4 . H, Table showing the reproducibility of the particles.

Uptake of T_4-liposome nanoparticles by astrocytes

Activated astrocytes uptake Cy5.5. Figure 8 shows Cy5.5, T_4-Cy5.5, Liposome- T_4-Cy5.5 uptake by activated astrocytes on different time point. (Fig 8E, upper panel: naïve, second panel: LPS(1ug/ml); third panel: Cy5.5; fourth panel: T4-Cy5.5; fifth panel: lipo-T4-Cy5.5.

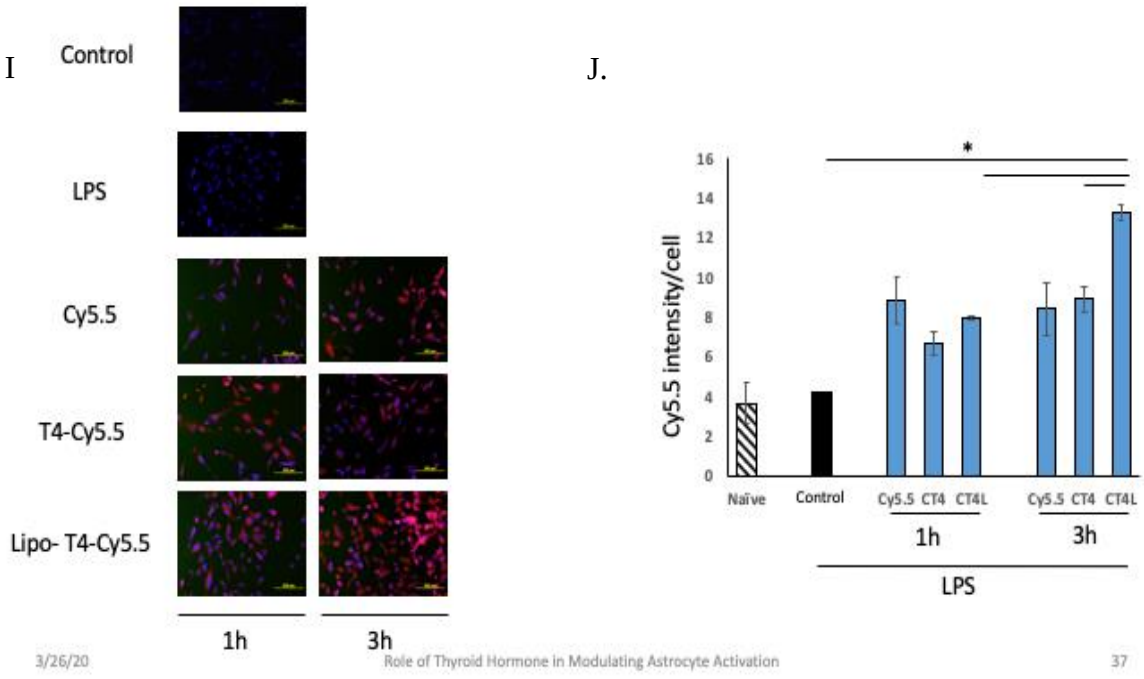


Figure 9. Astrocytes are activated by LPS

Representative immunofluorescence images showing Cy5.5, T_4-Cy5.5, Liposome- T_4-Cy5.5 uptake in astrocytes with LPS treatment. J, histograms showing imageJ quantitation of GFAP expression in astrocytes. Scale bar 200u, $^{**}p<0.01$, $^{***}p<0.001$.

Effect on cell viability

Different doses of T4 (50 nM,25 nM,12.5 nM) administered before LPS activation on astrocytes shows less viability whereas T4 treatment simultaneously with LPS and followed by LPS activation shows high viable. 25 nM T_4 dose shows significance. when administered simultaneously.

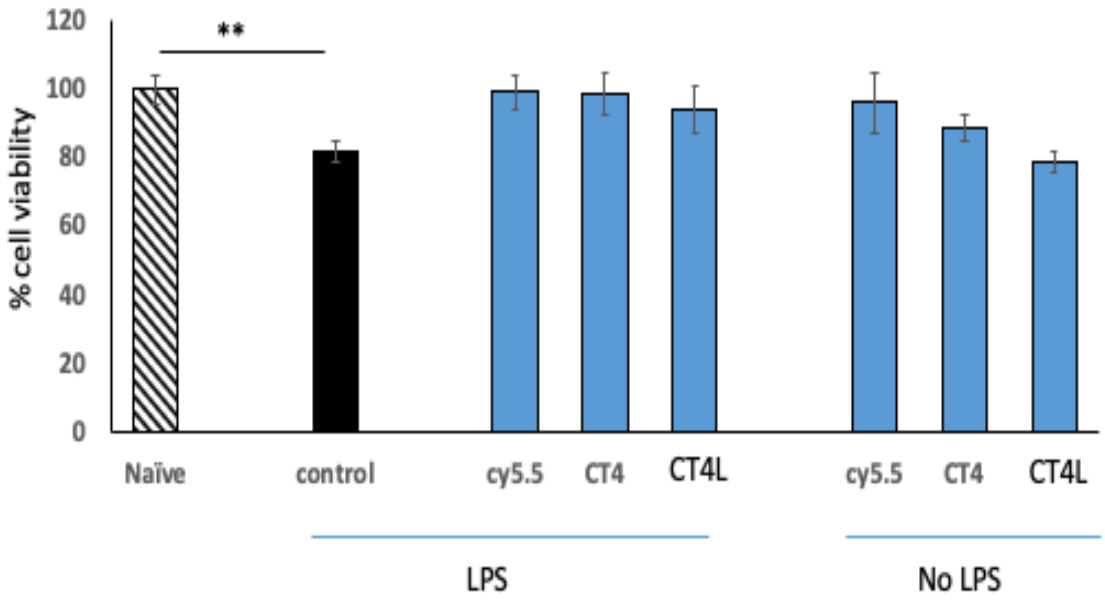


Figure 10. Cell viability of activated astrocytes when treated with T_4

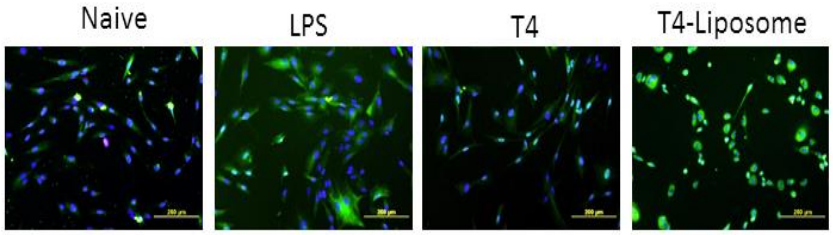
simultaneously with LPS, followed by LPS and before LPS treatment.

Representative histograms showing % cell viability of astrocytes. **p<0.01, ***p<0.001.

Effect on GFAP expression

Astrocytes expressed GFAP following activation by 1ug/ml LPS for 24 hours which was inhibited upon treatment with T4 and Liposome- T4-Cy5.5. GFAP expression changed significantly with T4 and Liposome- T4-Cy5.5 treatment (Figure 10).

K.



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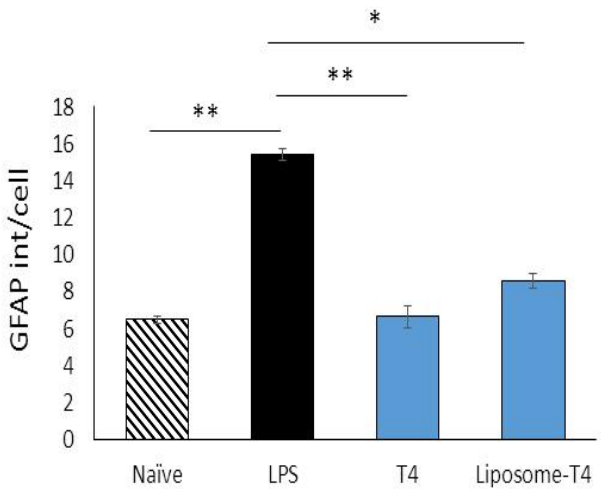

Figure 11. immunofluorescence images showing GFAP expression in astrocytes when T4 treatment

Chapter 4: Conclusions

LPS-activated astrocytes as a model of neurodegeneration**:** The Astrocyte dysfunction can lead to the precarious neuroinflammation that can propagate to neurodegeneration. Due to the compact structure of blood brain barrier it is very difficult for drugs to cross it. Astrocyte is the mediator to transport the nutrition, drugs to the neurons. The study shows that astrocytes are activated after 1ug/ml of LPS treatment for 24hrs. The GFAP expression shows significant activation of the cells without affecting viability of the cells**.**

Thyroid hormone as a major regulator of neuronal inflammation and homeostasis:

Thyroid Hormone is one of the major metabolic hormones of the human body. It possesses a huge role in cognitive impairment and neuronal homeostasis. In brain T_4 converts to T_3 with the help of astrocytes to its bio-active form. The study shows that 25 nM dosage of thyroxine is optimum for *in vitro* analysis. The Cy5.5 uptake concludes that it is taken up by the cells.

Thyroid hormone replacement therapy causes side effects:

Exogenous thyroid hormone is the best way to maintain homeostasis in deficit condition. Along with its therapeutic effect it exerts some adverse effects too. The half-life of oral supplement is very low. A novel therapy is desirable to maintain the requirement and also to release the drug slowly in the system.

References

Acosta, C., Anderson, H. D., & Anderson, C. M. (2017). Astrocyte dysfunction in Alzheimer disease. *J Neurosci Res, 95*(12), 2430-2447. doi:10.1002/jnr.24075

Burda, J. E., Bernstein, A. M., & Sofroniew, M. V. (2016). Astrocyte roles in traumatic brain injury. *Exp Neurol, 275 Pt 3*, 305-315. doi:10.1016/j.expneurol.2015.03.020

Fellin, T., Pascual, O., & Haydon, P. G. (2006). Astrocytes Coordinate Synaptic Networks: Balanced Excitation and Inhibition. *Physiology, 21*(3), 208-215. doi:10.1152/physiol.00161.2005

Grabbe, N., Kaspers, B., Ott, D., Murgott, J., Gerstberger, R., & Roth, J. (2020). Neurons and astrocytes of the chicken hypothalamus directly respond to lipopolysaccharide and chicken interleukin-6. *J Comp Physiol B, 190*(1), 75-85. doi:10.1007/s00360-019-01249-1

Hickman, S., Izzy, S., Sen, P., Morsett, L., & El Khoury, J. (2018). Microglia in neurodegeneration. *Nat Neurosci, 21*(10), 1359-1369. doi:10.1038/s41593-018-0242-x

Krebs, J. (2017). Implications of the thyroid hormone on neuronal development with special emphasis on the calmodulin-kinase IV pathway. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1864*(6), 877-882.

Maher, S. K., Wojnarowicz, P., Ichu, T.-A., Veldhoen, N., Lu, L., Lesperance, M., . . . Helbing, C. C. (2016). Rethinking the biological relationships of the thyroid hormones, l-thyroxine and 3,5,3′-triiodothyronine. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 18*, 44-53. doi:https://doi.org/10.1016/j.cbd.2016.04.002

Obregon, M. J., Escobar del Rey, F., & Morreale de Escobar, G. (2005). The effects of iodine deficiency on thyroid hormone deiodination. *Thyroid, 15*(8), 917-929. doi:10.1089/thy.2005.15.917

Ortiga-Carvalho, T. M., Chiamolera, M. I., Pazos-Moura, C. C., & Wondisford, F. E. (2016). Hypothalamus-Pituitary-Thyroid Axis. *Compr Physiol, 6*(3), 1387-1428. doi:10.1002/cphy.c150027

Ransohoff, R. M. (2016). How neuroinflammation contributes to neurodegeneration. *Science, 353*(6301), 777-783. doi:10.1126/science.aag2590

Seifert, G., Schilling, K., & Steinhäuser, C. (2006). Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat Rev Neurosci, 7*(3), 194-206. doi:10.1038/nrn1870

Sharp, D. J., Scott, G., & Leech, R. J. N. R. N. (2014). Network dysfunction after traumatic brain injury. *10*(3), 156.

Soloveva, M. V., Jamadar, S. D., Poudel, G., Georgiou-Karistianis, N. J. N., & Reviews, B. (2018). A critical review of brain and cognitive reserve in Huntington's disease. *88*, 155-169.

Sung, K., & Jimenez-Sanchez, M. (2020). Autophagy in astrocytes and its implications in neurodegeneration. *Journal of Molecular Biology*.

Thibaut, F. (2017). Neuroinflammation: new vistas for neuropsychiatric research. *Dialogues Clin Neurosci, 19*(1), 3-4.
Varela, M. L., Mogildea, M., Moreno, I., & Lopes, A. (2018). Acute Inflammation and Metabolism. *Inflammation, 41*(4), 1115-1127. doi:10.1007/s10753-018-0739-1
Zareh, M., Manshaei, M. H., Adibi, M., & Montazeri, M. A. (2019). Neurons and astrocytes interaction in neuronal network: A game-theoretic approach. *J Theor Biol, 470*, 76-89. doi:10.1016/j.jtbi.2019.02.024