Effect of hypothyroidism in metabolic profile of rats after cerebral ischemia/reperfusion: a 1H NMR study

L. Rastogi[1], S. N. Akhtar[2], and M. M. Godbole[3]

[1]Department of Endocrinology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, [2]Department of Microbiology and Centre of Biomedical Magnetic Resonance, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, [3]Department of Endocrinology, Sanjay Gandhi Post Graduate Institute, Lucknow, Uttar Pradesh, India

SYNOPSIS: Neuroprotective role of hypothyroidism is gaining attention in stroke. Changes in cerebra lactate, N-acetyl –L-aspartate (NAA) and other metabolites were determined by vivo ^{1}H MRS and infarct assessment by haematoxylin & eosin as well as triphenyltetrazolium chloride staining in fresh brain tissue after middle cerebral artery occlusion in euthyroid and hypothyroid rats. Compared to euthyroid ischemic reperfused rats, a significant decrease in infarct area and in ischemic neurons were observed in hypothyroid ischemic reperfused rats (H+I/R). Other metabolites were altered in H+I/R. NMR spectroscopy identified the metabolites involved in ischemia reperfusion and broaden the knowledge of molecules involved in neuroprotection by hypothyroidism .

INTRODUCTION: Brain ischemia initiates a cascade of metabolic events that lead to irreversible necrosis of neurons and glia. Insufficient oxygen delivery inhibits the synthesis of adenosine triphosphate, causing depletion of adenosine triphosphate and phosphocreatine and increases in inorganic phosphate and lactic. ^{1}H NMR permits measurement of lactate, which increases during anaerobic glycolysis, and therefore identifies brain ischemia. ^{1}H MRS can also be used to measure amino acids, including N-acetyl -L-aspartate (NAA) which is present predominantly in neurons and has been proposed as an MRS marker of neuronal density and viability Hypothyroidism results in hypo metabolic state and ischemia is a condition where tissue experiences less supply of blood .The beneficial effect of hypothyroidism is gaining attention, and it is reported that hypothyroidism is protective in stroke patients[1] and in animal model of stroke[2].The molecular mechanism of protection offered by hypothyroidism in stroke is not well defined. Understanding of molecules involved in protection by hypothyroid condition will give new dimensions to stroke therapy. The goal of this study was to examine metabolic differences between normal and hypothyroid rat brain and to determine ischemia/reperfusion changes using *In vitro* ^{1}H NMR spectroscopic at 400 MHz. In NMR spectroscopy, the ratios of the dominant peaks of the spectrum: N-acetyl aspartate (NAA), creatine, and lactate were compared. The metabolic response to reperfusion after temporary focal ischemia was compared with the histological findings.

MATERIAL AND METHODS: Forty male Sprague Dawley albino rats (weight 250-350g) were housed at the temperature of 25 ± 2°C with alternating 12 hour light and dark cycle and free access to standard food pellets and water. The rats were divided into two groups: euthyroid (E) and hypothyroid (H; 2-mercapto-1-methylimidazole 0.025% (w/v) for 6 weeks in drinking water). Thyroid hormone levels were measured by radioimmunoassay using kits from Diagnostic Product Company (New York, USA) in blood samples. Ten rats each from E & H group underwent ischemia/reperfusion experiments and termed as E+I/R and H+I/R group respectively. All animal procedures performed were in strict accordance with the institutional guidelines for animal care and research. Transient focal ischemia was induced by occluding middle cerebral artery (MCA) by advancing a 4.0 nylon filament for 2 hours [3] and retracting the same gently to allow the reperfusion for next 24 hours. Body temperature was maintained 36-38°C by using a heating lamp. All the animals were subjected to neurological evaluation by using a 6-point postural reflex test [4]. Tissue sampling was done 24 hours after reperfusion / sham operation in the entire group of rats at the time of sacrifice. Rats were anaesthetized and blood was collected through cardiac puncture for hormonal assay. Few rat were transcardially perfused for histopathology and other rats were rapidly dissected for infarct analysis.and was used for 2,3,5- tripheny tetrazolium chloride (TTC). For quantification of metabolite profiles in NMR spectroscopic studies, brain tissue metabolites extraction was done by 12 % perchloric acid. All ^{1} H- NMR experiments were performed on a Bruker Biopsin 400 MHz spectrometer at 25 ° C by suppressing the residual water signal by presaturation. Typical parameters used were spectral width: 8000Hz; data point: 32K; flip angle: 45 ° C; number of scans 64; relaxation delay 5 s and FT size: 32K. A reusable co- axial capillary containing calibrated quality of sodium salt of trimethylsilylpropionate (TSP) dissolved in deutrerium oxide was used for each NMR experiment to serve as reference.

RESULTS: Thyroid hormone levels were significantly decreased in rats treated with Methimazole confirming hypothyroid state. Significant decrease in neurological deficit, infarct area and in ischemic neurons was observed in H+I/R rats in comparison to E+I/R rats. ^{1} H- NMR spectra showed significant increase in lactate levels in E+I/R rats when compared to E. Lactate levels were significantly less in H+I/R rats when compared to E+I/R rats. N- acetyl- aspartate (NAA) and creatine levels were altered in both groups of rats.

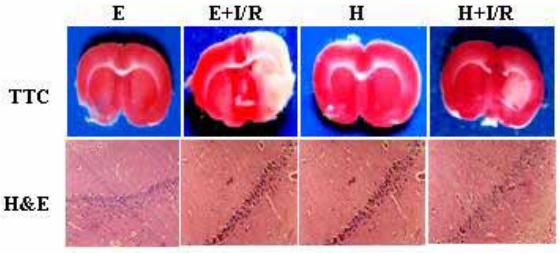

Fig 1: Representative TTC-stained coronal brain slices and H&E stained brain tissue of a euthyroid (E), hypothyroid (H) control rats and euthyroid (E+I/R) and hypothyroid (H+I/R) animals underwent 2 hour of MCA occlusion following 24 hour of reperfusion

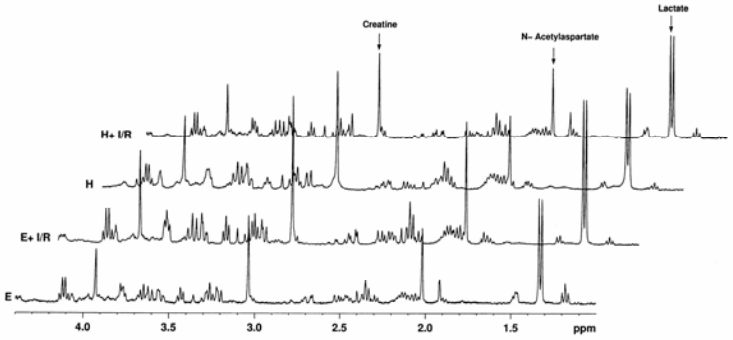

Fig 2: 1H-NMR spectra of PCA extracts of brain tissue metabolites of E, H control rats & E+I/R and H+I/R animals underwent 2 hour of MCA occlusion following 24 hour of reperfusion

DISCUSSION: An attempt is made here to identify the molecules involved in protection by hypothyroidism after Ischemia reperfusion injury. The rise in brain lactate that results from the mismatch between glycolysis and oxygen supply making it a hallmark for cerebral ischemia identification. In H+I/R rats, decrease in lactate levels were observed .NAA is exclusively confined to neurons in brain and its role in neuroprotection has been reported in neurodegenerative disease. Creatine is another substrate for energy production apart from glucose. Creatine and NAA are altered in H+I/ R rats. Our previous and current study shows the molecules involved in neuroprotection by hypothyroidism in ischemia reperfusion injury and the need of modulation of thyroid hormone (TH) levels in brain by TH antagonist or by any agent that create hypothyroxenemic state in brain *in vivo*. It can be beneficial as post ischemic treatments modality in stroke.

REFERENCES:

1. Alevizaki M, Synetou M, Xynos K, et al. Hypothyroidism as a protective factor in acute stroke patients Clinical Endocrinology 2006;65:369-372
2. Rastogi L, Godbole MM, Ray M, et al. Reduction in oxidative stress and cell death explains hypothyroidism induced neuroprotection subsequent to ischemia / reperfusion insult. Exp Neurol 2006: 200; 290-300.
3. Zea-Longa E, Weinstein PR, Carlson S, et al. Reversible middle artery occlusion without craniotomy in rats. *Stroke*1989; 20**:** 84-91.

ACKNOWLEDGMENT: This work was supported by research grants from the Council of Scientific & Industrial Research, Government of India to Leena Rastogi and Department of Science and Technology, Government of India to S N Akhtar.