Metabolic perturbations of Rat Spleen due to Chronic Cold Stress: 1H NMR based Metabolomic Study

SONIA GANDHI[1], HEMANTH KUMAR B S[1], SUNIL KOUNDAL[1], SHUBHRA CHATURVEDI[2], RAJENDRA P TRIPATHI[1], and SUBASH KHUSHU[1]
[1]NMR Research Centre, INMAS, DELHI, DELHI, India, [2]Division and Cyclotron & Radiopharmaceutical Sciences, INMAS, DELHI, India

Target Audience: Researchers, Clinicians and Students.

Purpose: Human beings have adapted to climatic extremes ranging from frigid arctic cold to enervating tropical heat. Thermal extremes can be endured by humans however there are demonstrable effects of exposure to abnormally high or low ambient temperatures which include physiological adaptation, changes in performance and influence on social behaviour[1,2]. The effects of cold stress can be detected in all aspects of body functioning. The prevention of cold stress is essential in protecting from multisystem stress. The cardiovascular and respiratory systems manifest the most obvious symptoms[3]. Hypoglycemia occurs, metabolic rate rises, unstable glucose levels can lead to further acidosis and neurologic changes[4]. Cold stress may result in immune suppression, gastrointestinal mucosal damage, affects central nervous system (CNS) & potential dysfunction to adrenal glands, kidney & lungs. These changes in metabolic activities of cells/tissues can directly be reflected in biological fluids & can be studied using NMR spectroscopy or LC-MS. However, NMR based tissue metabolic studies after cold exposure are almost negligible in literature.

Aim & Objective: To study metabolic perturbations & identify potential biomarkers in rat spleen tissue due to prolonged cold stress.

Material & Methods: 8-10 weeks old male Sprague Dawley rats (233±30g) were housed individually in stainless steel wire mesh cages. Room temperature & humidity were regulated at 24±1ºC & 40±10%, respectively. Rats were acclimatized in metabolic cages & they were divided into four groups, control (C, n=6), cold exposure for 1day (CE1, n=6), cold exposure for 5 days (CE2, n=6) and cold exposure for 15 days (CE3, n=6). On day 0, CE1, CE2 and CE3 groups were exposed to 4ºC for 8 hrs (9a.m. to 5p.m.). Animals were exsanguinated from cervical dislocation after 1, 5 & 15 days of cold exposure. Spleens were excised, washed with phosphate buffer and immediately snap frozen in liquid nitrogen. Polar metabolites from spleen tissues were extracted using perchloric acid & supernatant containing metabolites were lyophilized for 10-12 hrs and then dissolved in 600µL of deuterium oxide and transferred to 5mm NMR tube with 1mM TSP (in capillary) as an external reference compound for spectral acquisition. 1H NMR spectra were acquired on each sample at 400.13 MHz on a Bruker Avance 400 spectrometer at 298K using 1D ZGPR (RD-90°-Acq) pulse sequence. For each sample, 64 transients were collected into 32K data points with a relaxation delay (RD) of 2s, flip angle of 90° and a mixing period of 100ms. A spectral width of 9612Hz and an acquisition time per scan of 2.56s was used. Metabolites concentration was compared in C, CE1, CE2 & CE3 groups using one way ANOVA. Binning of each spectrum was carried out using Amix software, Bruker & Metaboanalyst[5] was used to carry out Principle Component Analysis (PCA).

Results & Discussion: Exposure to cold stress of 4ºC at different time point showed perturbations in endogenous metabolites. Visual comparison of ^{1}H NMR spectra of spleen tissue samples (Fig. 1) between control and cold exposure for 1, 5 and 15 days (8hrs each day) showed significant changes in metabolites such as taurine, choline, creatine, myo-inositol and aspartic acid which were further confirmed by loading plots (Fig. 2). Changes in taurine which is synthesized in pancreas via cysteine sulfinic pathway might indicate dysfunction in conjugation of bile acids, changes in antioxidant and osmoregulation properties, membrane destabilization or demodulation of calcium signaling. Myoinositol (an osmolyte) is an indicator of osmotic regulation. Changes in inositol level on cold stress indicate alterations in osmotic balance. Changes in creatine levels indicates altered glomerular filtration rate whereas on continuous exposure upto 15 days significant changes were observed. Significant changes in circulating creatine may also indicate increased biosynthesis, reduced muscle absorption, cell leakage or changes in reabsorption. Alterations in choline concentrations was also seen which might indicate changes in structural integrity and signaling roles for cell membranes. A 3-D PCA analysis (Fig. 3) showed time dependent, clear separation in the first principal component (PC) of the spleen tissue profile of rats exposed to cold stress for different time point. With the progression of cold exposure, rats showed higher metabolic changes on day 15 as compared to day 1 and could be easily separated from control indicating systemic metabolic variation due to cold exposure.

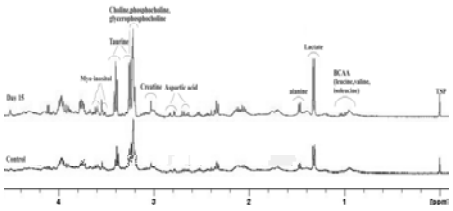

Fig 1: Comparison of ^{1}H NMR spectra of C & CE3 (day15) group spleen tissue samples showing increased intensity of metabolites with increased duration of cold exposure.

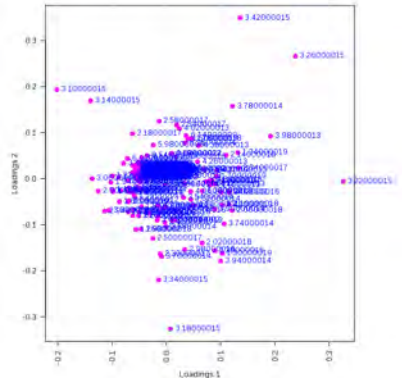

Fig. 2 Loading plots of ^{1}H NMR of spleen tissues of control, day1, 5 & 15 exposure groups representing chemical shift positions corresponding to metabolites altered due to cold stress.

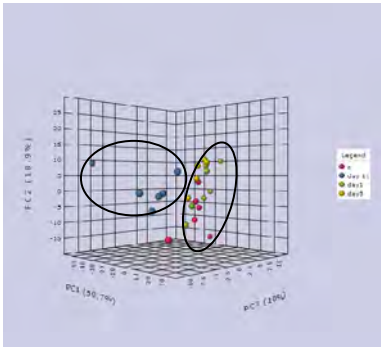

Fig 3: 3D PCA plots for spleen tissue samples showing distinct variation between control & cold stress groups (CE1, CE2 & CE3) with increased duration of cold exposure.

Conclusion: The present study indicates NMR based metabonomics in conjugation with statistical analysis serves as a powerful tool for non-invasive monitoring of various metabolic alterations in spleen tissue induced by prolonged cold stress. The changes might indicate osmotic imbalance, altered reabsorption, changes in cell integrity & signaling role for cell membrane. Present studies correlated with other biochemical, analytical & molecular parameters can be helpful in detecting early biomarkers for cold stress injuries in humans & organ specific dosimetry.

References:

1.Wang, X., Zhao, T., Qiu, Y., et al. **(2009)**. *J. Proteome Res*., 8, 2511–2518.
2.Ujita, J. **(1999)**. *J. Mol. Microbiol. Biotechnol*., 1, 243-255.
3. Machenbach et al **(1992).** *J. Epidemiol*., 46, 261
4.Blackburn, S. T.; Loper, D. L. **(1992)**. Maternal fetal & neonatal physiol., Philadelphia, WB Saunders, p 692.
5. Xia, J. MetaboAnalyst: a web server for metabolomic data analysis and interpretation, Nucleic Acids Res. **(2009)**. 37, W652-W660.