Stress induced alterations in the metabolic profile of human monocytes

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Introduction: Stress activates the hypothalamic-pituitary-adrenal-axis, inducing production of glucocorticoids such as cortisol, a known immuno-suppressant. The effect of glucocorticoids on immune function of T cells, macrophages etc., has been reported (1). However its direct effect on the metabolic profile of immuno-competent monocyte-macrophage series of cells has not been investigated. The aim of the study was to identify metabolite biomarker(s) of stress. Experiments were designed to assess the effect of dexamethasone, a synthetic glucocorticoid on the metabolic profile of THP1 cells; a human monocytic cell line differentiated *in vitro* to macrophages using PMA (Phorbol 12-myristate 13-acetate, PdTHP1). The metabolites generated by PdTHP1 macrophages cultured in the presence/absence of dexamethasone were monitored by high-resolution NMR spectroscopy. Twenty six metabolites were assigned in the proton NMR spectrum to establish the metabolome related to stress.

Materials & Methods: PdTHP1 cells were treated with dexamethasone at the physiological concentration (1μM,) and in excess (10μM) for 24 hrs. Metabolites were isolated by perchloric acid extraction method and lyophilized (2). ^{1}H NMR was carried out using 700 MHz (Agilent Technologies). One dimensional (1D) and two dimensional (2D) total correlation Spectroscopy (TOCSY) proton NMR spectrum were carried out to identify the metabolites. TSP (Sodium trimethyl-silyl-[2,2,3,3-H4] propionate) was added as an internal standard for chemical shift and quantification of metabolite concentration. The concentration of metabolites was determined by comparing the intensity of metabolite resonance obtained by integration of signal of interest with that of TSP

Results & Discussion: Twenty-six metabolites were identified by 1D and 2D TOCSY plots. Seven metabolites namely, Lactate, Alanine, Glutamine, Glutamate, Glutathione, Choline & Phosphorylethanolamine were significantly decreased in dexamethasone treated cells. Of the seven metabolites, lactate was decreased significantly at both concentrations, Alanine and Glutamate at 1μM concentration and Glutamine, Glutathione, Choline and Phosphorylethanolamine at 10μM concentration of dexamethasone. Three metabolites namely Acetate, Aspartate, and myo-inositol significantly increased in dexamethasone treated cells. Acetate was increased significantly at both concentrations, Myo-inositol at 1μM while Aspartate at 10μM concentration of dexamethasone. It has been reported that Glutamate, Glutamine, Choline were decreased in neural tissues during stress, whereas in human leukemic cell line and mouse thymoma cell line, choline and phosphorylethanolamine were decreased during stress (3-6). Cells treated with dexamethasone show multiple effects on cellular metabolism, ranging from the degradation of cell membrane which results in the decrease of choline levels; decrease in lactate levels which has been related to the reduced glycolysis; and changes in the phospholipid metabolismwhich leads to a decrease in phosphorylethanolamine concentration (5,7). The low concentration of glutamine limits the function of cells of immune system (4). In skeletal muscles under stress the release of glutamine exceeds its synthesis. This results in the lowering of intracellular glutamine concentration, enhanced rate of protein break down and amino acid metabolism (4). These metabolites associated with dexamethasone treated cells could be further assessed to serve as stress markers.

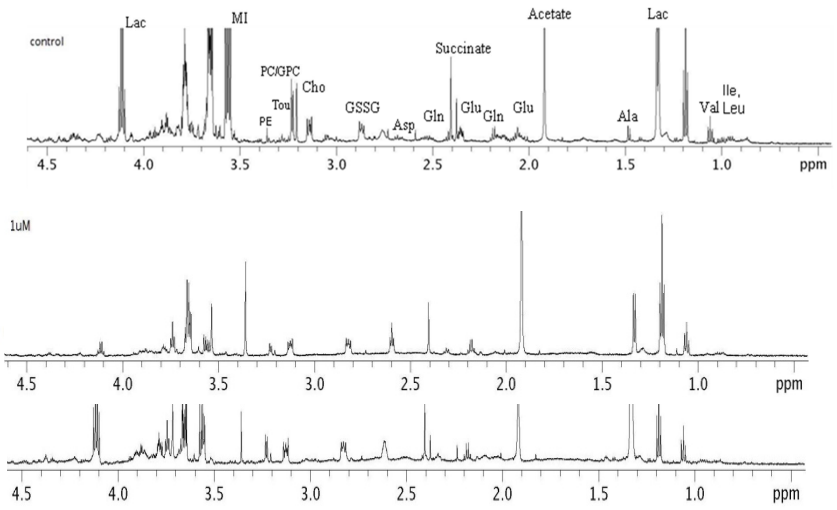

Figure: Comparative1D proton NMR Spectra of Control and Dexamethasone treated cells (1 & 10μM)

S.No.	Metabolites	Concentration (μmole) per million cells (Mean ± S.D)		
		Control (n=8)	1 μM Dexa (n=8)	10μM Dexa (n=8)
1	Lactate (Lac)	35.2 ± 22.6	9.7 ± 4.3*	15.6 ± 18.7**
2	Alanine (Ala)	2.2 ± 0.9	1.3 ± 0.6*	1.8 ± 0.7
3	Acetate (Ace)	21.1 ± 0.51	36.8 ± 7.4*	41.9 ± 8.8**
4	Glutamate (Glu)	10.0 ± 3.7	5.9 ± 1.5*	7.1 ± 1.7
5	Glutamine (Gln)	8.7 ± 3.3	6.7 ± 1.5	5.7 ± 1.0**
6	Aspartate (Asp)	9.6 ± 3.0	9.0 ± 4.5	15.4 ± 5.4**
7	Glutathione (GSSG)	14 ± 8.3	8.5 ± 2.9	5.5 ± 1.9**
8	Choline (Cho)	3.0 ± 1.4	1.7 ± 0.4	1.2 ± 0.3**
9	Phosphorylethanolamine (PE)	3.8 ± 0.6	1.8 ± 1.4	2.2 ±1.0**
10	Myo-Inositol (MI)	7.7 ± 1.6	31.2 ± 24.7*	6.6 ± 2.1

Table: Concentration of metabolites (μmole per million cells)
***P* < 0.05 – Control Vs 1 μM Dexa , ****P* < 0.05 – Control vs 10μM Dexa**

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