Università degli Studi di Pavia

Oxidative stress triggers an adaptive response in PBMC from Multiple Sclerosis patients and healthy subjects modulated by HSP70-2

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INTRODUCTION

Oxidative stress is involved in MS pathogenesis and progression by direct and indirect mechanisms of action, which also encompass the heat shock proteins 70 (hsp70s) family (Mansilla et al. , 2012). Intracellular hsp70s act as chaperones and anti-apoptotic proteins. Extracellular hsp70s process and present antigens, promoting the activation of immune system. Among the several proteins included in hsp70s family, the two major stress-inducible members (i.e. the Hsp70-1 and Hsp70-2) are encoded by HSPA1A and HSPA1B gene respectively, and the constitutively expressed non-inducible protein (i.e. Hsp70-hom) is encoded by HSPA1L gene (Brocchieri et al.,2008). These three genes are located on chromosome 6 (6p21.3), within the human leukocyte antigen (HLA) class III region.

We have described that HSP70-2 (rs1061581) polymorphism is related to the risk of develop MS in Caucasians (Boiocchi et al., 2014). The aim of the study is to investigate the effects of OS on mitochondrial activity in cultured peripheral blood mononuclear cells (PBMC) from MS patients and healthy donors, relating these aspects to rs1061581 genotype distribution.

METHODS

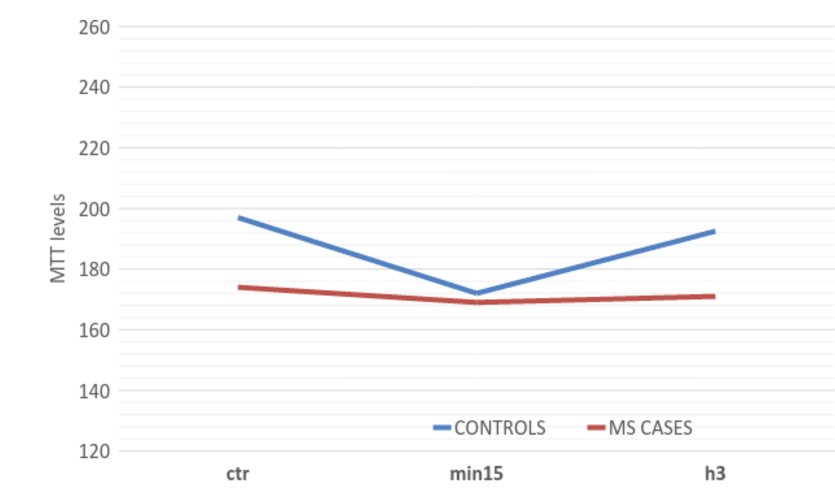
- PBMC cultures were treated with an OS stimulus (10 µM H_2O_2), and mitochondrial activity was evaluated by using MTT assay, at different time points (basal without treatment, 15 minutes and 3 hours after the stimulus).
- To test the differences between MTT time points we used the non-parametric Friedman test.
- Post hoc Wilcoxon tests with Bonferroni correction were used to compare median pairs when Friedman results were significant.

RESULTS

- We completed MTT concentration/response curves to H_2O_2 on PBMC for 49 MS cases and 46 controls and found a significant MTT variability over time points for both groups ($P<0.001$).
- Specifically, MTT basal levels of cases were significantly lower than controls ($P=0.03$), while did not differ any more 15 min after ($P>0.05$). Basal levels were almost restored for both groups 3 hours after H_2O_2 ($P>0.05$).
- We also stratified our analyses by rs1061581 genotype. Among MS cases, MTT basal levels of GG were higher w.r.t. MTT basal levels of AG and AA.
- The overall MTT variability over time points was significant for AA and AG subjects ($P<0.001$).
- Specifically, MTT significantly decreased considering 15 min time point w.r.t. the basal level (11 units reduction GG subjects and 6 units reduction AA and AG subjects) and increased at 3 hours only for GG and AG (10 units GG and 10 units AG). (Figure 1)

CONCLUSION

PBMC from MS patients showed lower basal mitochondrial activity compared to healthy subjects. OS in both groups induced a decrease in this activity, which is restored 3 hours after , suggesting MS patients PBMC are able to respond to OS . Moreover, rs1061581 G allele seems implicated in a better response to OS, suggesting Hsp70-2 can be important for the ability of PBMC to respond to OS. Understanding the modulation of Hsp70 in the complex scenario of MS is therefore of pivotal importance in order to develop, in the near future, further innovative and more suitable medical therapies.



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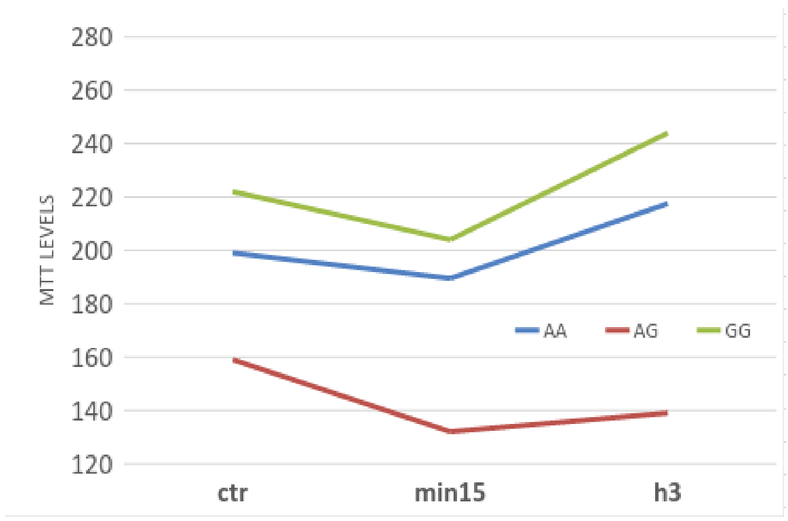

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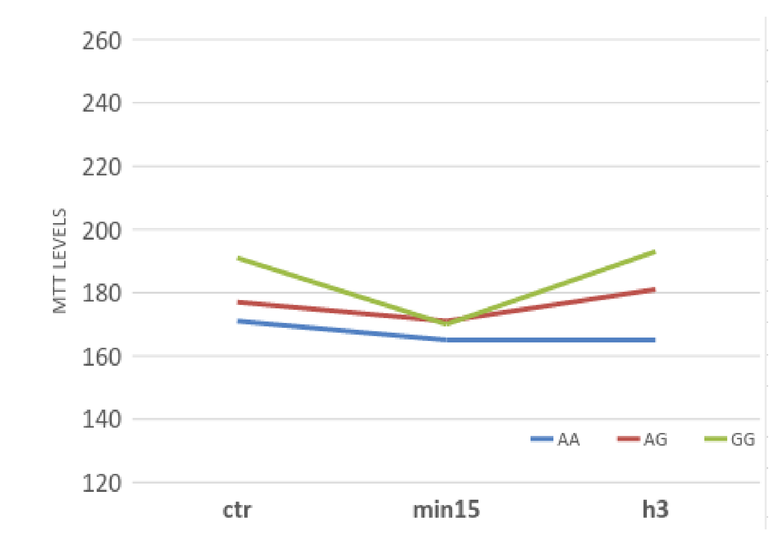

C

Figure 1

A: Comparison of median MTT levels concentration/response curves between MS cases and controls.

B-C:MTT variability by rs1061581 genotype in controls and MS cases.

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