

Introduction

Septic shock is the result of a hyper-inflammatory response by immune cells with pathologic “cytokine storm” largely modulated by the ubiquitin-proteasome pathway. Proteasomes regulate a number of cellular processes like aging, cell cycle, and inflammation [1,2]. The catalytic activities of proteasomes mainly reside in three subunits: X (β 5), Y (β 1) and Z (β 2) which correspond to chymotrypsin-like (CT), post-acidic (PA) and trypsin-like (T) activities, respectively. After priming with interferon- γ (IFN γ) or lipopolysaccharide (LPS); X (β 5), Y (β 1) and Z (β 2) subunits can be induced to LMP7 (β 5i), LMP2 (β 1i) and LMP10 (β 2i) immunoproteasome subunits. Several studies have shown the potential for targeting proteasome inhibition as drug therapy [3]. Previously our lab has identified the drug, resveratrol (Res), as a proteasome inhibitor in mouse macrophages, warranting further investigation on human monocytes.

Hypothesis

We hypothesize that the natural occurring drug, Res, is an inhibitor of LPS induced proteasome activity and inflammation by using human monocyte cell line (THP1).

Methods

THP1 cells (1×10^4 cells/ well) were treated with Res (10 μ M – 320 μ M) and incubated at 37 $^\circ$ C, 5% CO $_2$, for 30 min. Caspase-Glo reagent (100 μ L) was added to a total volume of 200 μ L/well. After 30 min, the relative luminescence units (RLU) were read according to manufacturer’s instructions (Promega, WI). For RT-PCR, THP1 cells were differentiated using PMA (phorbol-12-myristate-13-acetate) for 24 hrs, pre-treated with Res (80 μ M), followed by LPS. After 4 hrs, cells were centrifuged, and stored at -80 $^\circ$ C. RNA isolated using Qiagen RNeasy Mini kit and Taqman real-time RT-PCR was performed. Cell viability was measured using MTT assay by exposing THP1 cells to Res (0-100 μ M) for 24 hrs. All statistical procedures performed with GraphPad Prism 5 and presented as mean \pm SEM, $P < 0.05$.

Results

Effect of Resveratrol on proteasome activities (THP1 cells)

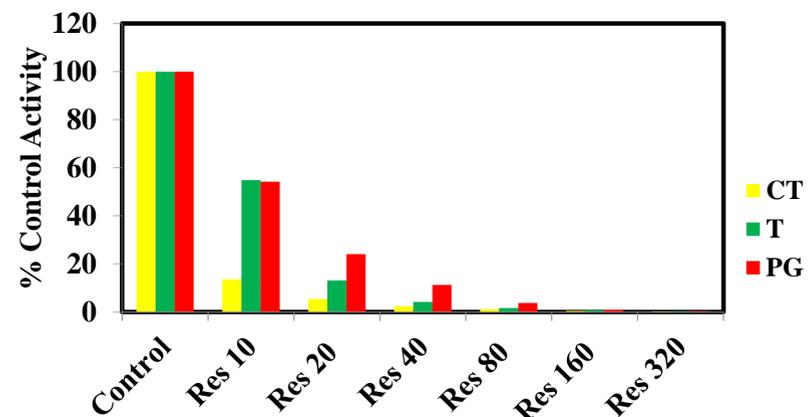


Figure 1. Resveratrol at 80 μ M almost completely blocked all three proteasome activities (chymotrypsin, trypsin and post glutamase).

Effect of LPS and Resveratrol on proteasome subunits-gene expression

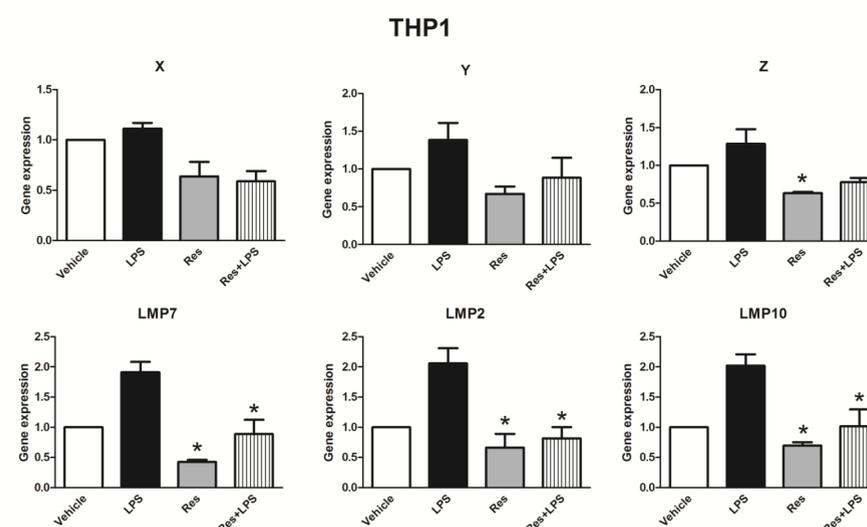


Figure 2: PMA differentiated THP1 cells were pretreated with resveratrol (80 μ M) and 0.4% DMSO for one hour and then treated with LPS (10 ng/ml) for 4 hrs. Real time RT-PCR was performed on RNA extracted from the treated cells for different proteasome subunits (n=2). LPS treatment results in upregulation of immunoproteasomes subunits and resveratrol is able to decrease the expression of immunoproteasomes. Data are shown as mean \pm SEM. *Statistically significant difference from LPS ($P < 0.05$).

Results

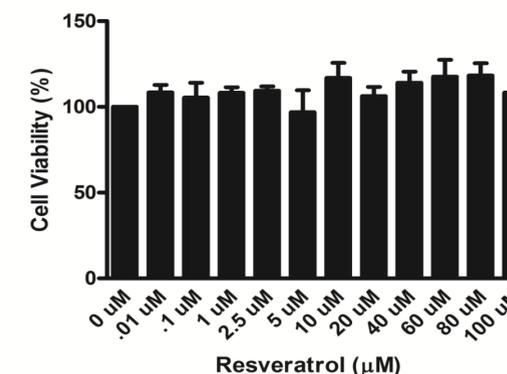


Figure 3a: THP1 cells pretreated with Res remained viable up to a drug dose of 100 μ M, as demonstrated by MTT assay results ($P > 0.05$; n=3)

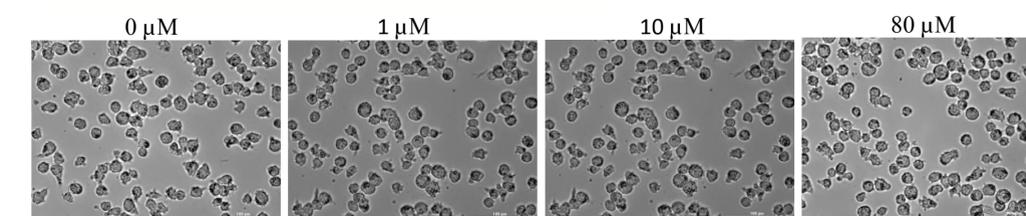


Figure 3b: Representative Bright field images of THP1 cells shown after exposure to varying concentrations of Res 0 μ M, 1 μ M, 10 μ M, and 80 μ M doses were well-tolerated without evidence of cell death.

Conclusion

Our data establishes that Res pretreatment reduced LPS-stimulated inflammatory response by downregulating immunoproteasome subunits as well as their activities, while limiting cell toxicity. Previous anti-sepsis drugs targeting only a single agonist have not been successful, presumably because multiple and potentially redundant sites and mediators are involved in sepsis. These findings implicate Res as a prospective, next-generation drug contributing towards the better understanding and management of a broad range of treatment resistant immune diseases, like septic shock.

References/Acknowledgements

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