SIRT-1 ACTIVATOR RESVERATROL PROTECTS HUVECs FROM HIGH GLUCOSE-DEPENDENT REDOX AND GLYCATIVE IMBALANCES

Mijiti M[1], Santini S Jr[1], Cordone V[1], Falone S[1], Amicarelli F[1]
[1] Dept. of Life, Health and Environmental Sciences, University of L`Aquila, Italy

Introduction

The link between diet and health is very interesting, in that it is ascertained that modifications in dietary intake may be important in causing, preventing or delaying the onset of several dysfunctions and diseases [1]. According to national Diabetes Statistics Report, worldwide diabetic population is sharply increasing [2]. Endothelial dysfunctions represent an early-occurring alteration in obesity- and diabetes-related disorders [3,4]. Both redox imbalances and buildup of methylglyoxal (MG), a glycolysis-derived pro-oxidant compound which is a major precursor of advanced glycation end-products, are thought to be causative factors for the onset of many hyperglycemia-induced tissue damages occurring in diabetes [5]. Stress response to pro-oxidants, DNA repair, and mitochondrial function is crucially regulated by Sirt2-related NAD-dependent protein deacylases (sirtuins), among which SIRT1 and SIRT3 plays a critical role in cellular and mitochondrial homeostasis [6]. SIRT1 is known to be activated by reserveratrol (RSV), a polyphenolic antioxidant compound found in red wine, grapes, and other foods [7]. Several relevant studies reported that RSV aroused a prolonged beneficial effect on the endothelial redox balance by modulating expression of key antioxidant enzymes [8-9]. However, very limited knowledge is available about how RSV may limit or prevent high-glucose imbalances in redox status and anti-glycation defences within human endothelial cells. In particular, no literature data is available as to whether SIRT1 is required by RSV to counteract oxidative and glycative stress in HG-challenged endothelial cells.

Hypothesis and objective(s)

Hypothesis: In the present study, which is a part of a wider work, we hypothesized that redox-active diet-derived RSV may prevent or limit the pro-oxidant and pro-glycation events that are triggered by hyperglycemia on human endothelial cells [10-11], possibly by activating sirtuin 1 (SIRT1)-dependent pathways (Fig. 1).

Resveratrol (RSV)

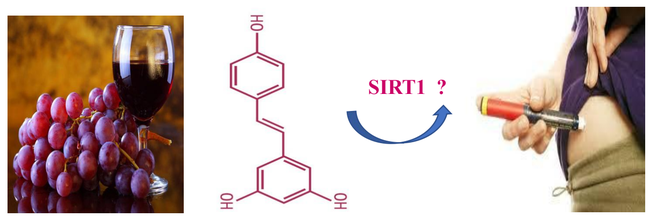

Figure 1. Scheme of hypothesis on RSV and Diabetes

Objective: to demonstrate that RSV is able to protect human umbilical vein endothelial cells (HUVECs) from redox impairment and glycation-related processes that are induced by high glucose (HG) treatment.

Materials

HUVECs

HUVECs were purchased from Lonza (C2519A (pooled donor)).

High glucose (HG)

HG was purchased from Sigma-Aldirich (G7021-100G).

Resveratrol (RSV)

RSV was provided from Sigma-Aldirich (R5010-100MG).

Experimental methods

Cell amplification	HUVECs were amplified until 3~4 passages.
Cell treatments	Cells treated with HG and/or RSV
Biochemical assays	Protein expression: 1) SIRT1, SIRT3 2) Glyoxalase 1 (GLO1), glyoxalase 2 (GLO2) (Fig. 5) 3) Catalase (CAT), Nuclear factor erythroid 2-related factor 2 4) (NRF2), Superoxide dismutase 2 (SOD2) (Fig. 6)

Results

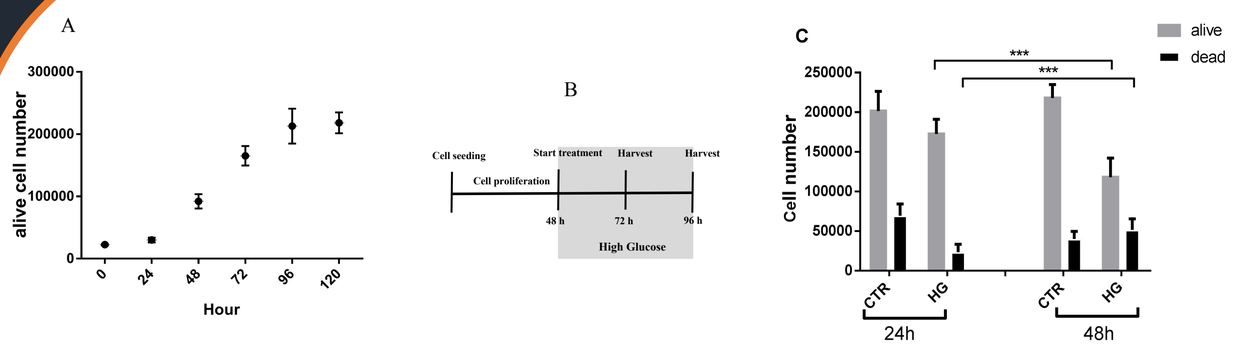

Figure 2. A): HUVECs growth curve. Data was expressed as means ± s.d. (n=4); B) Scheme of the treatment with HG; C) HUVECs treatment with high glucose (25mM) neither 24 or 48 hours, two-way ANOVA was performed for data analysis, ***P<0.01, n=4.

The cell growth curve showed that three distinct phases (lag phase, exponential phase, stationary phase) of HUVECs proliferation were clearly observed [Fig. 2A]. On this basis, we decided to start all the treatments after 48 hours from seeding, which corresponded to the exponential phase [Fig. 2B]. Moreover, we found that cells were incubated with 24-h HG tended to show less cytotoxicity, as compared to 48-h HG incubation [Fig. 2C].

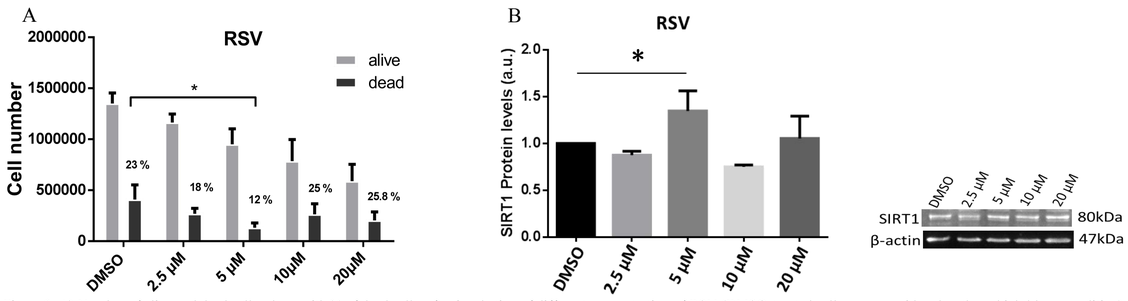

Figure 3. A) Number of alive and dead cells, along with % of dead cells, after incubation of different concentration of RSV (DMSO-treated cells were considered as the vehicle/sham condition). Data was expressed as means ± s.d. (n=4), t-test, P<0.05; B) SIRT1 protein level was normalized against β-actin, Data was expressed as arbitrary units (means ± s.d.) (n=2, exception of 5 µM RSV, n=8, respectively). *P<0.05, one-way ANOVA followed by post-hoc Tukey tests for multiple comparison was applied.

Our results revealed that treatment with 5 µM RSV tended to induce less cell death, as compared to other concentrations [Fig. 3A]. Moreover, the treatment with 5 µM RSV in normal glucose condition caused the overexpression of SIRT1 protein [Fig. 3B], and this was confirmed by all subsequent experiments.

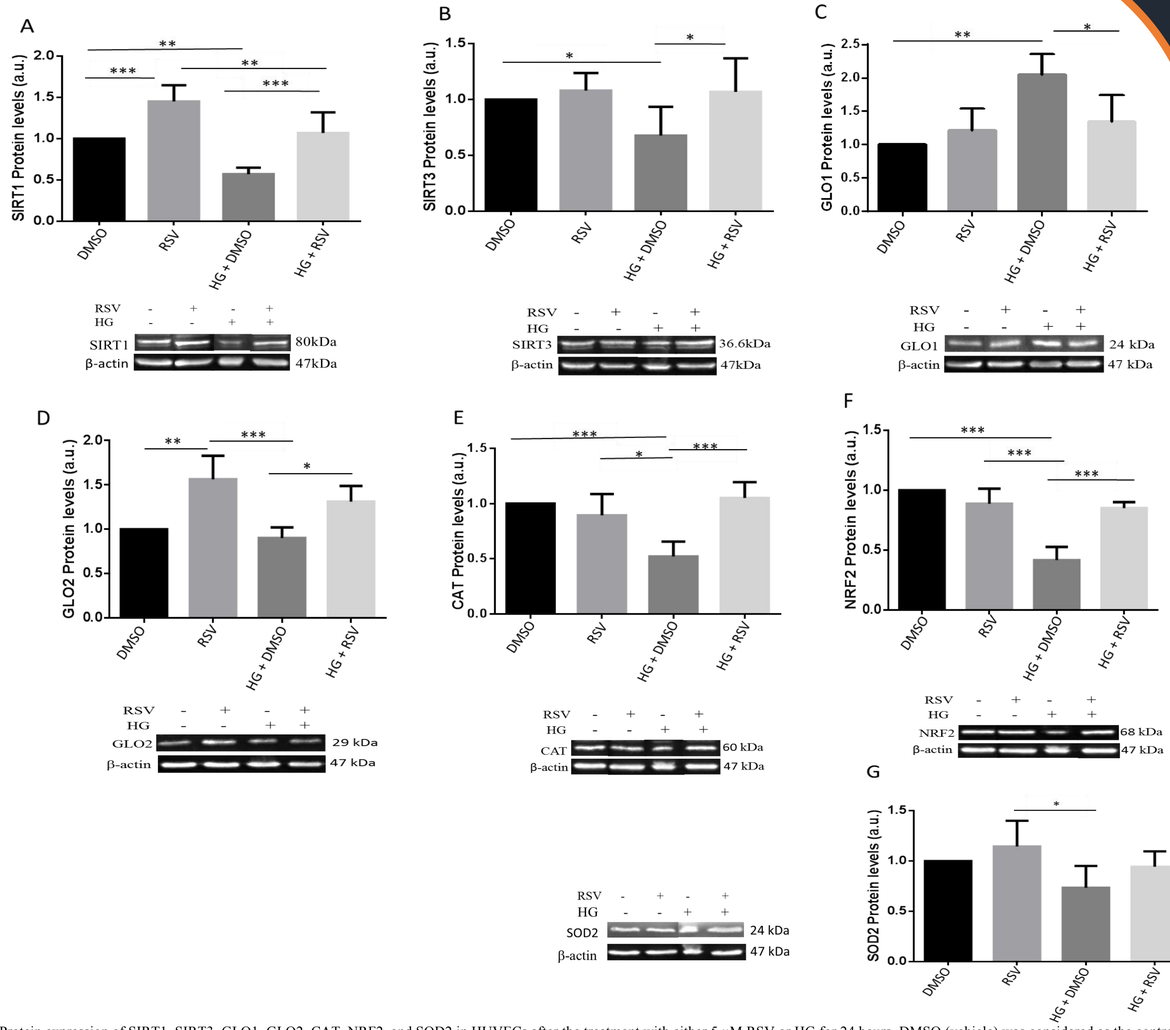

Figure 4. Protein expression of SIRT1, SIRT3, GLO1, GLO2, CAT, NRF2, and SOD2 in HUVECs after the treatment with either 5 µM RSV or HG for 24 hours. DMSO (vehicle) was considered as the control condition. Protein expression was normalized against that of β-actin. Data was expressed as arbitrary units (means ± s.d.) (n=4 for all panels, with the exception of panels A and B (n=7 and n=6, respectively)). *P<0.05, **P < 0.01, (2 × 2 factorial ANOVA followed by post-hoc Tukey tests for multiple comparison).

The levels of SIRT1, SIRT3, CAT, and NRF2 were significantly down-regulated by HG [Fig. 4A, B, E, and F, respectively]. Interestingly, RSV completely abolished such a negative effect of HG, thus restoring the normal protein levels of SIRT1, SIRT3, CAT and NRF2. Conversely, GLO1 was found to be greatly increased in cells treated with HG, and this effect was totally reverted by RSV treatment [Fig 4C]. RSV significantly increased GLO2 level in HG condition compared to HG+DMSO [Fig 4D]. Finally, we observed nearly significant reduced SOD2 protein expression after HG treatment, which was reverted by co-incubation with RSV (not shown). However, in order to confirm this preliminary result, we are currently replicating SOD2-targeting western blotting.

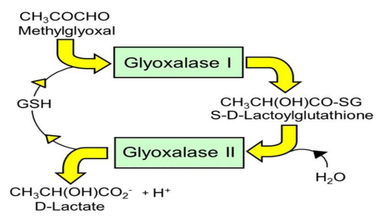

Figure 5. Glyoxalase system [12]

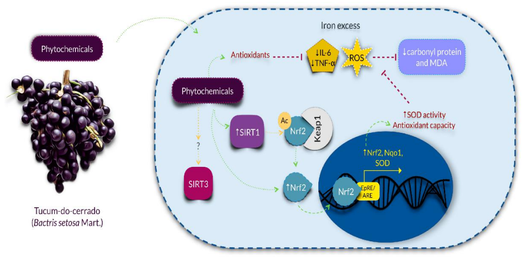

Figure6. Phytochemical via SIRT1-NRF2 mediate antioxidant pathway [13]

Conclusion and Future direction

Overall, our results suggest that the expression of the major determinants of antioxidant and antiglycative defence systems in human endothelial cells was impaired by HG. Interestingly, we found that all the HG-induced imbalances were reverted by co-incubation with resveratrol. In particular, we obtained interesting clues that RSV may ameliorate the hyperglycemia-induced pro-oxidant and pro-glycation stress on HUVECs possibly by regulating SIRT1–dependent pathway (Fig.7). Nevertheless, further studies are needed to elucidate the underlying mechanisms.

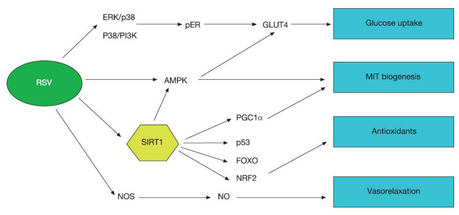

Figure 7. Schematic signalling pathways induced by resveratrol [14]

In the future, we will investigate how RSV may have an impact on the transcriptional levels of the major antioxidant and antiglycative gene products, both in normoglycemic and hyperglycemic conditions.

Reference

1. Holmboe-Ottesen G, Wandel M. Changes in dietary habits after migration and consequences for health: a focus on South Asians in Europe. Food & Nutrition Research. 2012;56:10.3402/fnr.v56i0.18891. doi:10.3402/fnr.v56i0.18891.
2. http://www.deathtodiabetes.com Slides from Death to Diabetes Workshop: Impact of Diabetes is devastating: physically, emotionally, financially, worldwide.
3. Schmitt.CA, Heiss.EH, Dirsch.VM. Effect of resveratrol on endothelialcell function: Molecular mechanisms. Biofactors. 2010 Sep-Oct;36(5):342-9.
4. Rajendran P, Rengarajan T, Thangavel J, et al. The Vascular Endothelium and Human Diseases. International Journal of Biological Sciences. 2013;9(10):1057-1069. doi:10.7150/ijbs.7502.
5. Negre-Salvayre A, Salvayre R, Augé N, Pamplona R, Portero-Otín M. Hyperglycemia and glycation in diabetic complications. Antioxid Redox Signal. 2009;11(12):3071-3109
6. Kim, Seok-Jo et al. "The Role of Mitochondrial DNA in Mediating Alveolar Epithelial Cell Apoptosis and Pulmonary Fibrosis." Ed. Jaime M. Ross and Giuseppe Coppotelli. International Journal of Molecular Sciences 16.9 (2015): 21486–21519. PMC. Web. 8 Feb. 2018.
7. Borra MT, Smith BC, Denu JM. Mechanism of human SIRT1 activation by resveratrol. J Biol Chem. 2005;280(17):17187-95.
8. Gordish KL, Beierwaltes WH. Resveratrol induces acute endothelium-renal vasodilation mediated through nitric oxide and reactive oxygen species scavenging. American Journal of Physiology-Renal Physiology. 2014;306(5): F542-F550. doi:10.1152/ajprenal.00437.2013.
9. Lubrano V, Balzan S. Enzymatic antioxidant system in vascular inflammation and coronary artery disease. World Journal of Experimental Medicine. 2015;5(4):218-224. doi:10.5493/wjem.v5.i4.218.
10. Cordone V., Falone S., Santini S. Jr, Di Tomo P., Di Silvestre S., Mandatori D., Formoso G., Amicarelli F., Pandolfi A. 2017. Characterization of dicarbonyl stress and redox balance in HUVEC from women with gestational diabetes. Conference on Insulin and Exercise Signalling for Glucose Homeostasis and Metabolic Health. 6-8 September. University of Bath, UK.
11. Falone S., Di Tomo P., Santini S. Jr, Di Silvestre S., Cordone V, Mandatori D., Formoso G., Amicarelli F., Pandolfi A. 2016. Caratterizzazione dello stress dicarbonilico e del bilancio redox in colture di cellule endoteliali umane derivate da cordoni di donne affette da diabete gestazionale. 26th National Congress of the Italian Society of Diabetology (S.I.D.). 4-7 May, Rimini, Italy.
12. Rabbani N, Thornalley PJ. Glyoxalase Centennial conference: introduction, history of research on the glyoxalase system and future prospects. Biochem Soc Trans. 2014;42(2):413-8.
13. Marcela de Sa Barreto da Cunha, Sandra Fernandes Arruda. Tucum-do-Cerrado (Bactris setosa Mart.) May Promote Anti-Aging Effect by Upregulating SIRT1-Nrf2 Pathway and Attenuating Oxidative Stress and Inflammation. Nutrients 2017, 9(11), 1243; doi:10.3390/nu9111243.
14. E. Rodríguez-Bies, S. Santa-Cruz Calvo, P. Navas, G. López-Lluch Resveratrol: an ergogenic compound. Rev Andal Med Deporte 2009;2:12-8.

Contact Information

Name : Mahmut MIJIT
Rep-Eat Project, Univeristy of Teramo,
Email: Mmijiti@unite.it
Website:*www.rep-eat.unite.it*
Phone: 3312891607

We gratefully acknowledge financial support from

This project has received funding from European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713714