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Current Trends in Metabolomics

Research Article

Lara RC, et al. Curr Tr Metabol: CTM-101.
DOI: 10.29011/CTM-101/100001

Oxidative Stress: *In Vitro* Comparative Evaluation of the Resveratrol Modulator Capacity in Neuro 2-A Lines and Human Leukocyte Cells

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Citation: Lara RC, Araújo GR, de Mello AP, Leal TR, Abreu de Melo MI, et al. (2018) Oxidative Stress: *In Vitro* Comparative Evaluation of the Resveratrol Modulator Capacity in Neuro 2-A Lines and Human Leukocyte Cells. Curr Tr Metabol: CTM-101. DOI: 10.29011/CTM-101/100001

Received Date: 22 June, 2018; **Accepted Date:** 13 July, 2018; **Published Date:** 20 July, 2018

Abstract

The aging of the world's population is a challenging reality. Biological aging begins at birth and continues to death. Several mechanisms have been reported as causes of this evolutionary process, such as: oxidative stress, telomere wear, hormonal dysregulation and immunosenescence. Among the first, oxidative stress has been one of the most accepted to explain aging and death. Antioxidants are compounds capable of inhibiting or slowing down the oxidation of biomolecules and act to protect against oxidative damage. The present study aims to evaluate the protective and modulating comparative effects of Resveratrol on Neuro 2-A and Human Leukocyte cells (donors aged 50-80 years old) in the aging process. The Neuro 2-A cell line was cultured by appropriate methods; as well as the recruitment of donors for venipuncture and subsequent isolation of leukocytes was performed through established protocols. For the evaluation of the production of reactive oxygen species, the chemiluminescence luminol dependent test of luminol was used. Nitric Oxide production was verified by the Griess reaction. The results showed that 5 μM Resveratrol was able to significantly decrease the production of Reactive Oxygen Species in Human Leukocytes, increase the production of Nitric Oxide in Neuro 2-A and Human Leukocytes and decrease the formation of peroxynitrite in the same immune and neural cells as mentioned above. Polyphenols exhibit various biological activities, including the prevention of oxidative stress common in atherosclerosis and aging. Our findings suggest that: Resveratrol behaves as an intervener to combat reactive species; neuro and protective immune, since it increases the production of nitric oxide, previously diminished by the oxidative environment to which the cells were exposed. It is also an efficient antioxidant in the fight against oxidative stress at the peripheral level - immune cells and central - neural cells, reducing the formation of peroxynitrite.

Keywords: Aging; Human leukocytes; Hydrogen peroxide; Neuro 2-A; Nitric oxide; Oxidative stress; Peroxynitrite; Resveratrol; ROS

Introduction

Global aging is a challenging reality of this century. In 2010, there were 576 million people aged 65 years old and over, worldwide. The expectation for 2050 is that this number will triple, reaching 1.5 billion people [1].

Aging can be defined as the gradual biological impairment of normal body functions accompanied by a decreased ability to respond to stress and by a greatly increased risk of morbidity and mortality. This complex and multi-factorial process is characterized by a progressive failure in maintaining tissue homeostasis with a consequent direct impact on the functional ability of organs and eventually of the entire organism that causes a significant loss of fitness. The process of aging is recognized as the dominant risk factor for most human diseases; including cancer, cardiovascular diseases and neurodegenerative disorders that ultimately lead aged organisms to death [2,3].

Aging is a time-related process of functional deterioration at cellular, tissue, organelle, and organismal level that ultimately

brings life to end. Cellular senescence, a state of permanent cell growth arrest in response to cellular stress, it is believed to be the driver of the aging process and age-related disorders [4]. Thus, senescence corresponds to a complex phenotype of biology that manifests itself in all systems and organs, affecting the physiology, thus exerting an impact on the functional capacity of the individual to the one that is more susceptible to chronic diseases [5].

Thus, it becomes crucial to understand aging mechanisms. The process of aging involves different interdependent hallmarks on cellular, molecular and organ level [6].

The mitochondrial free radical theory of aging, later termed the Oxidative Stress Theory (OST), is currently one of the most popular correlative theories of the aging process. OST explains aging at the molecular level and results from failure to maintain oxidative defences, mitochondrial integrity, proteostasis, barrier structures, DNA repair, telomeres, immune function, metabolic regulation, and regenerative capacity [7].

Free radicals are highly reactive atoms or molecules with one or more unpaired electron(s) in their external shell and can be formed when oxygen interacts with certain molecules. These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants. The terms Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) refer to reactive radical and non-radical derivatives of oxygen and nitrogen, respectively. ROS and RNS are produced by all aerobic cells and play an important role in aging process as well as in age-related diseases. ROS and RNS generation is not only limited to determine deleterious effects but it is also involved in the extraction of energy from organic molecules, in immune defense, and in the signalling process [8]. Incomplete reduction of oxygen leads to the generation of different species of free radicals, such as: superoxide anion($O2^{\bullet-}$), hydroxyl(•OH) and hydrogen peroxide(H_2O_2) the latter being the main cause of cellular oxidative damage and exogenous senescence inducer) [6,9].

Besides their detrimental effects, low to moderate doses of ROS are beneficial and essential to cellular signalling and response to infectious agents [10]. Therefore, it is important to keep a balance of redox homeostasis with the help of redox-modulators/anti-oxidants within the cell, either by enzymes that convert ROS to less dangerous species (e.g., superoxide dismutase) or by anti-oxidant molecules that scavenge ROS in a non-specific manner (glutathione, thioredoxin). However, the appropriate pro-oxidant/anti-oxidant balance in a normal cell can be shifted towards the pro-oxidant state called OST, when the production of oxygen species is increased greatly or when the levels of anti-oxidants are diminished. Exogenous anti-oxidants can be categorized as natural and synthetic [11].

Antioxidants are compounds capable of inhibiting or slowing the oxidation of biomolecules and the ability to protect against oxidants. They can be enzymatic or non-enzymatic, being produced endogenously or not. Among non-enzymes, include polyphenols, in situ, foods, vegetables and fruits [12].

Resveratrol (RSV) (3,5,4'-trihydroxy-trans-stilbene) a phytoalexin naturally synthesized or induced in plants (particularly grape skin, cranberries, blueberries and red wine), is a widely known antioxidant and anti-inflammatory agent and it has been shown to reverse age-associated pathologies in small mammals [13,14].

RSV is a natural polyphenol primarily found in plants protecting them against pathogens, as well as harmful effects of physical and chemical agents. In higher eukaryotic cells and organisms, this compound displays a remarkable range of biological activities, such as anti-oxidant, anti-inflammatory, anti-cancer, anti-aging, cardio- and neuro-protective properties [13,14].

In view of this scenario, the present study aims to evaluate the protective and modulating comparative effects of Resveratrol on Neuro 2-A and Human Leukocyte cells (donors aged 50-80 years old) in the aging process.

Methodology

Cellular Culture of Neuro 2-A Lineage

The Neuro 2-A cell line (ATCC CCL131) (N2-A), derived from spontaneous albino mouse tumour, was purchased from the Cell Bank of Rio de Janeiro(Brazil). For culturing, they were placed in sterile 75 cm^2 growth bottles containing Dulbecco's Phosphate Buffered Saline(DMEM) culture medium. It was added 10% (v/v) foetal bovine serum. The bottles were incubated in an oven at 37°C humidified with 5% carbon dioxide (CO_2). The medium was replaced every two or three days, according to the confluence of the cell monolayer (observed under inverted microscope – MOTIC AE21) and subcultures (passages). When the bottles reached 80% confluence, the medium was aspirated, and the cell monolayer washed twice with calcium-free and magnesium-free Phosphate Buffer Solution (PBS). Subsequently, 5 mL of trypsin solution (0.20% trypsin solution and 0.02% EDTA) was used to detach the monolayers. After detachment, the cells were withdrawn from the flask, placed in Falcon tube with 5 mL of DMEM, and centrifuged at 1500 rpm for 10 minutes. After centrifugation, the supernatant was removed, and the pellet re-suspended in 1 ml DMEM. The cells were then counted with 0.3% Trypan Blue in the Neubauer chamber (OPITIK LABOR).

Selection of Individuals for Donation of Blood Cells

Individuals between 50 and 80 years old, of both sexes, who were in a normal aging process, were selected. For the selection of donors, we adopted exclusion criteria according to the SENIEURS protocol [15], which is a system of study in which a healthy individual is differentiated from the unhealthy, and presents exclusion criteria; diseases and medicines that influence the immunogenic parameters. The exclusion criteria indicated in the protocol are: smokers, patients with infection, inflammation, lipo-

proliferative disorders, arteriosclerosis, heart failure, use of drugs that directly influence the immune function. Those who were able to participate were submitted to the signing of a Free and Informed Consent Term (FICT). Consubstantiated Opinion of the Ethics and Research Committee of the Federal University of Minas Gerais: 1.171.758.

Venous Peripheral Blood Collection and Separation of Human Leucocytes

After reading and signing TCLE, peripheral blood was obtained by venipuncture from healthy adult volunteers using tubes containing heparin as anticoagulant14 and the leukocytes were isolated according to the technique described by Bicalho et al. [16].

Briefly

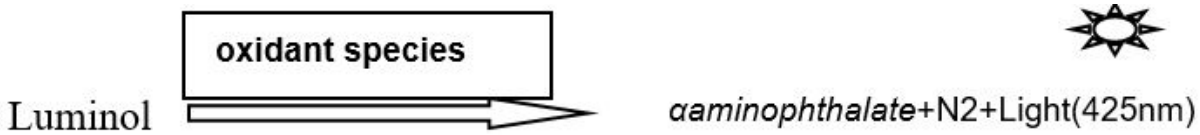
4 mL of heparinized blood was added over 3 mL of Leucopaque gradient (density = 1.13) in glass tubes. After adequate centrifugation two distinct phases separated by a ring of leukocytes were obtained. These were packed in a siliconized tube, which had its volume filled with PBS for two washing sessions. Subsequently, the Human Leucocytes (HL) were suspended in 1.0 ml of DMEM. The final volume was adjusted to 1×10^6 cells in 100 µL.

Cell Viability Test for Trypan Blue Incorporation

For the accomplishment of the experiments of cytotoxicity, the number of viable cells was estimated with the use of Trypan Blue dye. The basis of this method is based on the microscopic observation that viable cells are impermeable to this dye, whereas non-viable cells have permeability; due to the formation of pores in the membrane. In this way, non-viable cells exhibit blue staining after treatment [17]. For this test, the Trypan Blue dye (0.3%) was added and the cells counted in Neubauer chamber. For the experiment to be considered valid, the equivalent samples should have at least 75% viable cells.

Chemiluminescence Assay

The cells produce a natural luminosity defined as native or natural chemiluminescence. However, this luminosity can be amplified by using chemical reagents that, when reacted with ROS produced, emit the amplified luminescence, according to the reaction below:



This technique is based on the reaction between luminol (5-amino-2,3-dihydro-1,4-phthalozinedione) and the reactive species generated in the absence or presence of the different concentrations of the substances H_2O_2 and RSV, individually.

Chemiluminescence in N2-A and HL Cells

A total of (5×10^3 N2-A cells) and (1×10^6 HL) were pre-incubated in siliconized tubes for 20 minutes with 30% v/v H_2O_2, temperature of 37°C and 5% CO_2 to mimic an environment of oxidative stress, while cells from the control and RSV groups were stimulated with incubated PBS alone. After the 20 minutes incubation, both cells were washed with PBS and centrifuged for 3 minutes at 1500 rpm, where all the supernatant was removed, and the cell pellet was re-suspended in 200 µL of DMEM. The cells were stimulated with 5 µMRSV and were incubated for 24 hours. After these 24 hours, cells were centrifuged at 1500 rpm and re-suspended in 100 µL of DMEM for the chemiluminescence procedures. In specific tubes for Luminometer were placed the 100 µL of cells, 10 µL of 10^{-4} M luminol and PBS to complete the final volume of 700 µL. Each tube was placed in the Luminometer and the reading was performed in 15-minute runs. The experiments were done in triplicate.

Production of Nitric Oxide by Griess Reaction in N2-A and HL Cells

Quantification of nitrite according to Griess Reaction [18] was performed to observe the production of Nitric Oxide (NO) by N2-A cells and HL using the following protocol: A total cell (5×10^3 N2-A) (1×10^6 HL) were used in a final volume of 200 µl – 96well plate- of DMEM culture medium (plus 10% foetal bovine serum). They were incubated in a humidified oven at 37°C and 5% CO_2 for 24 hours. After that time, the supernatant was removed and in the specific wells 30% H_2O_2 v/v was added for 20 minutes at 37°C and 5% CO_2 to mimic an oxidative stress environment. After this time the cells were washed with PBS and the specific wells were stimulated with 5 µM RSV. The plate was incubated again at 37°C and 5% CO_2 for 24 hours. Subsequently, the supernatant was collected and used for nitrite dosing. The pellet was re-suspended in 200 µl of DMEM and immediately subjected to cell viability analysis. For nitrite dosing, supernatant was used, which were arranged in 96 well plates. The supernatants were added 100 µl Griess solution (1% sulfanylamine in 2.5% phosphoric acid and 0.1% naphthylenediamine in 25% phosphoric acid, 1:1 ratio). The contents of the plate were analysed by microplate reader, at wavelength 540 nm. The nitrite concentration was calculated by linear regression using the standard curve obtained from a solution of 1 mM sodium nitrite and DMEM culture medium. This experiment was performed in triplicate for each sample and repeated for 3 times.

Production of Peroxynitrite in N2-A and HL Cells

The peroxynitrite($ONOO^-$) quantification was evaluated ac-

cording to Hughes & Nicklin's [19] technique to observe $ONOO^-$ production by N2-A and HL using the following protocol: both cell types were plated in 6 wells culture plates at 1×10^6 cells/well in a final volume of 2 ml of DMEM culture medium (plus 10% foetal bovine serum). They were incubated in a humidified oven at 37°C and 5% CO_2 for 24 hours. After that time the supernatant was removed and 30% v/v H_2O_2 was added and continuously incubated for 30 minutes to mimic an oxidative stress environment in the specific wells. After this time the cells were washed with PBS and the specific wells were stimulated with 5 µM RSV. The plate was incubated again at 37°C and 5% CO_2 for 16 hours. After this incubation period, the supernatant was discarded and 2 mL of ultrapure water was added to the cell pellets and vortexed for 1 minute. Such content was measured and quantified in a plate spectrometer at wavelength of 302 nm.

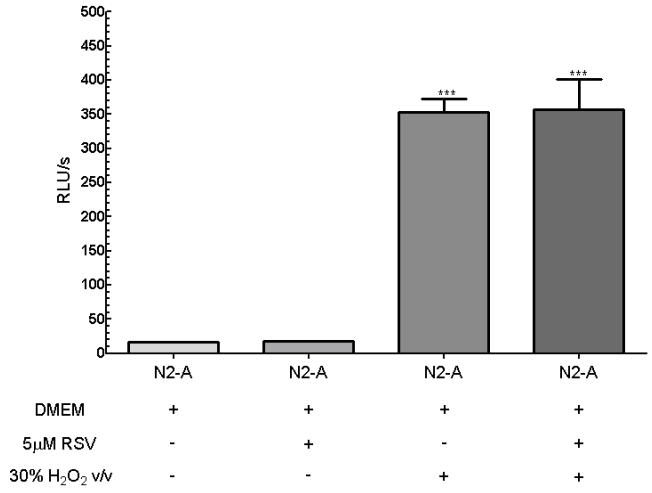
Statistical Analysis

The experiments were submitted to statistical analysis by the program GraphPad Prism Program 5.

Results

Comparative Evaluation by Chemiluminescence of the Effect of RSV on the Production of ROS Induced by H_2O_2 in N2-A and HL Cells

In view of the certification that 30% v/v H_2O_2 promotes an oxidative stress environment in N2-A and HL both remain viable, the power of RSV in modifying the production of ROS generated by the stressor stimulus. The results obtained can be observed in Figure 1.



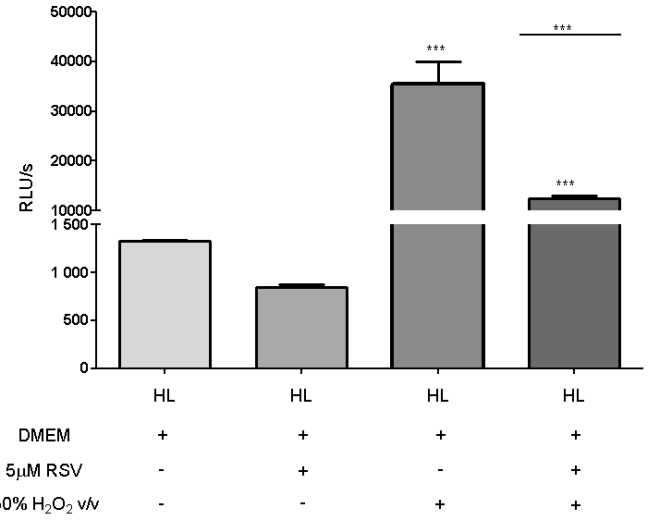


Figure 1: Comparative chemiluminescence evaluation of the effect of RSV on the production of ROS induced H_2O_2 in N2-A and HL. Panel A= Generation of ROS in N2-A expressed in RLU/min (Relative Light Unit) Panel***p <0.0001 = significant result by Kruskal-Wallis test, followed by Dunn's post-test. B=Generation of ROS in HL expressed in RLU/min***p <0.0001 = significant result by ANOVA test, followed by Bonferroni's post-test.

The experiments were done in triplicate with a final number of 45 readings. Analysing Figure 1 (Panel A), we noticed that RSV was not able to alter the profile of increased ROS production by N2-A, caused by their exposure to H_2O_2. In contrast, Figure 1 (Panel B) shows an opposite profile. That is, RSV as an isolated stimulus to HL decreased the basal ROS production exhibited by these cells. In addition, it has markedly decreased the ROS exacerbation seen when these blood cells were exposed to H_2O_2.

Comparative Evaluation of the Production of NO in N2-A and HL Cells in the Environment of Oxidative Stress and the Involvement of the RSV

In order to quantify the production of nitric oxide (NO-another important component of oxidative stress/aging), Griess method [18] was used for N2-A and HL. The ability of RSV to alter NO production was evaluated in the presence of a stressor stimulus. The results are shown in Figure 2.

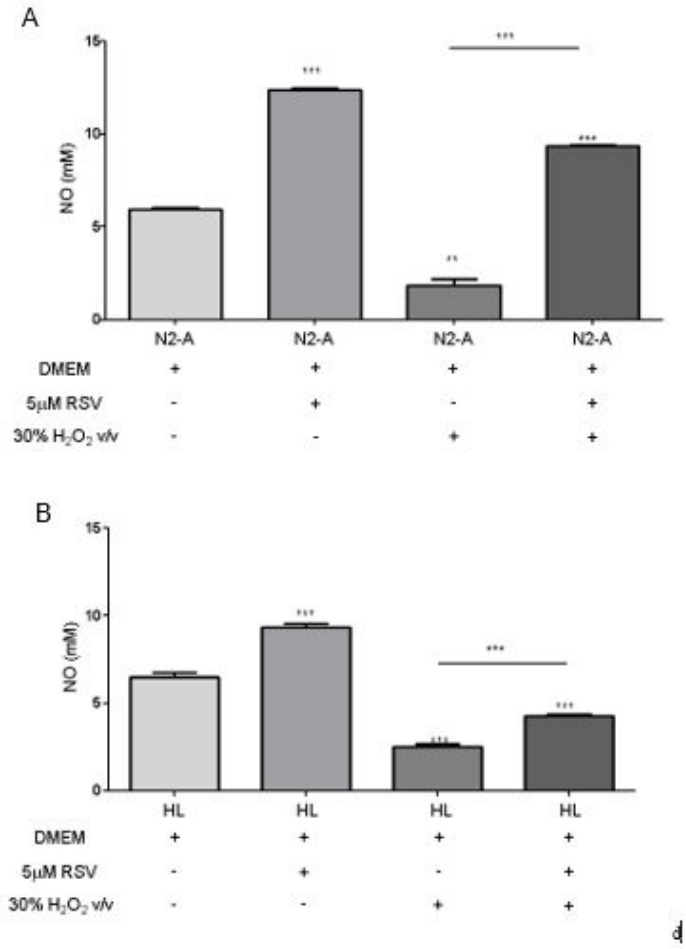


Figure 2: Comparative effect of RSV on the production of NO in N2-A and HL exposed to H_2O_2. Panel A= Generation of NO in N2-A expressed in mM Panel B= Generation of NO in HL expressed in mM***p <0.0001 = significant result by ANOVA test, followed by Bonferroni's post-test.

The experiments were done in triplicate with a final number of 45 readings. Analysing Panels A and B of Figure 2, it can be seen that the profile for both N2-A and HL against the proposed stimuli were the same. The antioxidant increased NO production by both cells, compared to baseline cellular quantification of this reactive species. And, it was able to change the NO decrease profile displayed when neural and blood cells were stimulated by H_2O_2. In conclusion, RSV acted to increase NO production by N2-A and HL.

Comparative Evaluation of ONOO- Production in N2-A and HL cells in the Environment of Oxidative Stress and the Involvement of the RSV

In order to quantify the production of ONOO- by N2-A and HL and the role of RSV in an oxidative stress environment, the technique postulated by Hughes & Nicklin was used [19]. The ability of RSV to alter the production of ONOO- in the presence of H_2O_2 was evaluated. The results are shown in Figure 3.

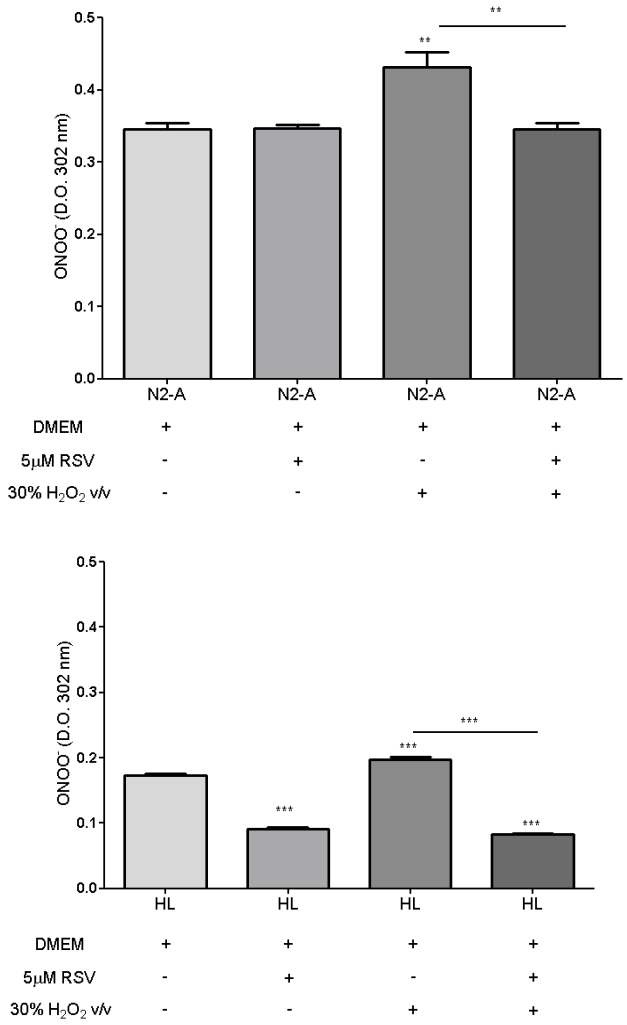


Figure 3: Comparative effect of RSV on ONOO- production in N2-A and HL exposed to H_2O_2. Panel A= Generation of $ONOO^-$ in N2A expressed in D.O. Panel B= Generation of ONOO- in HL expressed in D.O.***p <0.0001 = significant result by ANOVA test, followed by Bonferroni's post-test.

The experiments were done in triplicate with a final number of 45 readings. Analysing Figure 3 (Panel A), we noticed that RSV was able to decrease in 20% of $ONOO^-$ production by N2-A, caused by their exposure to H_2O_2. In contrast, Figure 3 (Panel B) illustrates that RSV as an isolated stimulus to HL decreased in 47.57% basal $ONOO^-$ production exhibited by these cells. In addition, it markedly decreased in 58.24% the exacerbation of $ONOO^-$ seen when these blood cells were exposed to H_2O_2.

Discussion

The causes of aging are due to multi-factorial processes. It is well known that aging is associated with inflammation, NF-κB activation, increased apoptosis, impaired mitochondrial activity, and a decline in immune function, as well as increased free-radical production rate [20].

N2-A cell clone from a spontaneous albino mouse neuroblastoma originated from spinal cord cells is a widely used lineage in neuronal models *in vitro* [21]. It shows neuronal phenotype both morphologically and neurochemically [22]. These neuronal cells have also been studied for the evaluation of antioxidants, mitochondrial activity and oxidative [23].

H_2O_2 originates from the enzymatic or spontaneous dismutation of superoxide anions, which are the by-products of a wide and ubiquitous variety of oxidases. Because of its high membrane permeability, intracellularly formed hydrogen peroxide induces deleterious intracellular effects and in neighbouring cells. Although H_2O_2-induced toxicity has been observed in different cell types, neurons are particularly vulnerable to H_2O_2-induced toxicity [24].

The data supporting the important role of oxidative stress in the aging process and neurodegenerative diseases support the beneficial effect of antioxidants as adjuvant therapy. This is because, the brain does not present fully efficient antioxidant defenses, making it vulnerable to the damage of this oxidative stress and deficient in preventing oxidative diseases with the senescence of the organism. The levels of glutathione and antioxidant enzymes are much lower in the Central Nervous System, compared to erythrocytes and peripheral tissues [25-27].

A recent study showed that the administration of RSV decreases nuclear-factor kappa B (NF-κB) and reduces the pro-inflammatory and pro-oxidant status associated with aging, which restores the function of the affected pancreas of aged SAMP8 (mice-accelerated-senescence prone, short-lived mice) [28-30].

Chaves et al. [31,32] demonstrated in granulocytes an increase in ROS from the age of 40 and RNS from the age of 50 years. Concomitantly with this increase, these authors observed a significant decrease in antioxidant power, suggesting a metabolic cellular imbalance in aging. Our results suggest that in N2-A, RSV failed to behave as an antioxidant. It was not effective in reducing the production of ROS (augmented by exposure to H_2O_2).

In contrast, in immune cells - HL -, RSV was able to sharply and sharply decrease the production of ROS that had been increased by exposure to H_2O_2. These results are in agreement with the findings described by BAARINE et al. [33] in cardio myocytes; where such polyphenol was able to protect the cardiac cells from the harmful effects of H_2O_2.

NO is an important signalling molecule with multiple pivotal roles in the cardiovascular and neural systems, as well as in inflammatory response [34]. (NO is an important molecular messenger in the brain and plays an important role in learning and memory and functions as a neuroprotective agent [35].

Findings of Simão & cols, which is RSV positively regulates NO production by signalling pathways together PI3-kinase/Akt (phosphatidylinositol 3-kinases/protein kinase B) and ERK (kinase-regulated extracellular signalling) [36]. These data provide a mechanistic basis for a potential application of RSV in therapy for neurovascular repair.

The results obtained show that RSV is able to increase NO production in both cell types of our study (N2-A, HL), a highly positive finding that corroborates the study by Xia et al., which points out that part of the protective effects of RSV are attributable to eNOS-derived NO. RSV enhances NO bioavailability by stimulating NO production and by preventing superoxide-mediated NO breakdown and it still plays an important role in learning and memory, which should by no means be neglected when we talk about aging [37].

The $ONOO^-$, potent oxidant, is formed by $O2^-$ and NO, on a base controlled by the diffusion rate of both radicals [38]. A generation of $ONOO^-$ *in vivo* can lead to the oxidation and nitration of lipids, DNA and proteins [39].

Immune cells, including circulating mononuclear cells, can reach the CNS as part of the immune surveillance process [40]. Our results in both N2-A and HL cells show that RSV was able to decrease the $ONOO^-$ synthesis in such neural and immune cells, evidencing the neuroprotective and immunomodulatory capacity of the polyphenol in question.

Such findings are seen against the literary: Studies by Brito et al. showing that RSV could block oxidation of LDL by $ONOO^-$ and by Olas et al. showing that RSV reduced peroxynitrite-mediated oxidation and nitration of serum proteins and platelet proteins led us to examine the $ONOO^-$ scavenging activity of RSV [41].

Conclusion

The results of our study showed that 5 μM RSV concentration was able to: decrease the production of reactive oxygen species in HL, increase the production of nitric oxide in N2-A and HL that had been decreased by exposure to hydrogen peroxide. It also, decrease the formation of $ONOO^-$ which is a highly damaging molecule to the organism in both studied cell types.

Our findings suggest that: RSV behaves as an intervener to combat reactive species; neuro and protective immune, since it increases the production of NO, previously diminished by the oxidative environment to which the cells were exposed. It is also an efficient antioxidant in the fight against oxidative stress at the peripheral level - immune cells and central - neural cells, reducing the formation of $ONOO^-$.

Acknowledgements

FAPEMIG APQ- 02574-14, CNPq, CAPES, PRPq - UFMG and IVC Health- testes em saúde humana LTDA supported this paper.

References

1. Chaves AC, Fraga VG, Guimarães HC, Teixeira AL, Barbosa MT, et al. (2017) Estrogen receptor-alpha gene XbaI A > G polymorphism influences short-term cognitive decline in healthy oldest-old individuals. Arq Neuropsiquiatr 75: 172-175.
2. Nicolai S, Rossi A, Di Daniele N, Melino G, Annicchiarico-Petruzzelli M, et al. (2015) DNA repair and aging: the impact of the p53 family. Aging (Albany NY) 7: 1050-1065.
3. Aramillo Irizar P, Schäuble S, Esser D, Groth M, Frahm C, et al. (2018) Transcriptomic alterations during ageing reflect the shift from cancer to degenerative diseases in the elderly. Nat Commun 9: 327.
4. Bu H, Wedel S, Cavinato M, Jansen-Dürr P (2017) MicroRNA regulation of oxidative stress-induced cellular senescence. Oxidative Medicine and Cellular Longevity 2017: 2398696.
5. Teixeira IN, Guariento ME (2010) Biology of aging: theories, mechanisms, and perspectives. Cien Saude Colet 15: 2845-2857.
6. Höhn A, Weber D, Jung T, Ott C, Hugo M, et al. (2016) Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. Redox Biol 11: 482-501.
7. Chandrasekaran A, Idelchik MDPS, Melendez JA (2017) Redox control of senescence and age-related disease. Redox Biol 11: 91-102.
8. Liguori I, Russo G, Curcio F, Bulli G, Aran L, et al. (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13: 757-772.
9. Vitale G, Salvioli S, Franceschi C (2013) Oxidative stress and the ageing endocrine system. Nat Rev Endocrinol 9: 228-40.
10. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, et al. (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39: 44-84.
11. Gülçin İ (2012) Antioxidant activity of food constituents: an overview. Arch Toxicol 86: 345-391.
12. Schaffer TK, Wohlenberg MF, Medeiros N, Martins JB, Agostini F, et al. (2016) Evaluation of antioxidant activity of grapevine leaves extracts (*Vitis labrusca*) in liver of Wistar rats. An Acad Bras Cienc 88: 187-196.
13. Bauer ME (2005) Stress, glucocorticoids and ageing of the immune system. Stress 8: 69-83.
14. Rashtbari S, Dehghan G, Yekta R, Jouyban A, Iranshahi M (2017) Effects of Resveratrol on the Structure and Catalytic Function of Bovine Liver catalase (BLC): Spectroscopic and Theoretical Studies. Adv Pharm Bull 7: 349-357.
15. Ligthart GJ, Corberand JX, Fournier C, Galanaud P, Hijmans W, et al. (1984) Admission criteria for immunogerontological studies in man: the SENIEUR protocol. Mech Ageing Dev 28: 47-55.
16. Mániková D, Šestáková Z, Rendeková J, Vlasáková D, Lukáčová P, et al. (2018) Resveratrol-Inspired Benzo[b]selenophenes Act as Anti-Oxidants in Yeast. Molecules 23: 507.
17. Esmann L, Idel C, Sarkar A, Hellberg L, Behnen M, et al. (2010) Phagocytosis of apoptotic cells by neutrophil granulocytes: diminished proinflammatory neutrophil functions in the presence of apoptotic cells. J Immunol 184: 391-400.
18. Bicalho HMS, Gontijo CM, Nogueira-Machado JA (1981) A simple technique for simultaneous human leukocytes separation. Journal of Immunological Methods 40: 115-116.
19. Konopka K, Pretzer E, Felgner PL, Düzgüneş N (1996) Human immunodeficiency virus type-1 (HIV-1) infection increases the sensitivity of macrophages and THP-1 cells to cytotoxicity by cationic liposomes. Biochim Biophys Acta 1312: 186-196.
20. Griess JP (1864) XVIII. On a new series of bodies in which nitrogen substituted for hydrogen. Philosophical Transactions of the Royal Society of London 154: 667-731.
21. Hughes MN, Nicklin HG (1968) The chemistry of pernitrites. Part I. Kinetics of decomposition of pernitrous acid. Journal of the Chemical Society A: Inorganic, Physical, Theoretical 450-452.
22. Ginés C, Cuesta S, Kireev R, García C, Rancan L, et al. (2017) Protective effect of resveratrol against inflammation, oxidative stress and apoptosis in pancreas of aged SAMP8 mice. Exp Gerontol 90: 61-70.
23. Calderón FH, Bonnefont A, Muñoz FJ, Fernández V, Videla LA, et al. (1999) PC12 and neuro 2a cells have different susceptibilities to acetylcholinesterase-amyloid complexes, amyloid25-35 fragment, glutamate, and hydrogen peroxide. J Neurosci Res 56: 620-631.
24. Basta-Kaim A, Budziszewska B, Jaworska-Feil L, Tetich M, Kubera M, et al. (2006) Antipsychotic drugs inhibit the human corticotropin-releasing-hormone gene promoter activity in neuro-2A cells-an involvement of protein kinases. Neuropsychopharmacology 31: 853-865.
25. Amazzal L, Lapôtre A, Quignon F, Bagrel D (2007) Mangiferin protects against 1-methyl-4-phenylpyridinium toxicity mediated by oxidative stress in N2A cells. Neurosci Lett 418: 159-164.
26. Konyalioglu S, Armagan G, Yalcin A, Atalayin C, Dagci T (2013) Effects of resveratrol on hydrogen peroxide-induced oxidative stress in embryonic neural stem cells. Neural Regen Res 8: 485-495.
27. Karuppagounder SS, Pinto JT, Xu H, Chen HL, Beal MF, et al. (2009) Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. Neurochem Int 54: 111-118.
28. Floyd RA, Hensley K (2002) Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. Neurobiol Aging 23: 795-807.
29. Chalimoniuk M, Jagsz S, Sadowska-Krepa E, Chrapusta SJ, Klapcinska B, et al. (2015) Diversity of endurance training effects on antioxidant defenses and oxidative damage in different brain regions of adolescent male rats. J Physiol Pharmacol 66: 539-547.
30. Liu Z, Wu Z, Li J, Marmalidou A, Zhang R, et al. (2017) Protective effect of resveratrol against light-induced retinal degeneration in aged SAMP8 mice. Oncotarget 8: 65778-65788.
31. Martins Chaves M, Rocha-Vieira E, Pereira dos Reis A, de Lima e Silva R, Gerzstein NC, et al. (2000) Increase of reactive oxygen (ROS) and nitrogen (RNS) species generated by phagocyting granulocytes related to age. Mech Ageing Dev 119: 1-8.

32. Chaves MM, Costa DC, Souza DA, Lima e Silva Fd, Machado JA (2008) Correlation between ROS production and InsP3 released by granulocytes from type 1 diabetic patients in a cAMP-dependent manner. Curr Aging Sci 1: 51-55.

33. Baarine M, Thandapilly SJ, Louis XL, Mazué F, Yu L, et al. (2011) Pro-apoptotic versus anti-apoptotic properties of dietary resveratrol on tumoral and normal cardiac cells. Genes Nutr 6: 161-169.

34. Modun D, Giustarini D, Tsikas D (2014) Nitric Oxide-Related Oxidative Stress and Redox Status in Health and Disease. Oxid Med Cell Longev 2014: 129651.

35. Thatcher GR, Bennett BM, Reynolds JN (2006) NO chimeras as therapeutic agents in Alzheimer's disease. Curr Alzheimer Res 3: 237-245.

36. Simão F, Pagnussat AS, Seo JH, Navaratna D, Leung W, et al. (2012) Pro-angiogenic effects of resveratrol in brain endothelial cells: nitric oxide-mediated regulation of vascular endothelial growth factor and metalloproteinases. J Cereb Blood Flow Metab 32: 884-895.

37. Xia N, Förstermann U, Li H (2014) Resveratrol and endothelial nitric oxide. Molecules 19: 16102-16121.

38. Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552: 335-344.

39. Barbosa LF, de Medeiros MHG, Augusto O (2006) Danos oxidativos e neurodegeneração: o quê aprendemos com animais transgênicos e nocautes? Química Nova 29: 1352-1360.

40. François A, Julian A, Ragot S, Dugast E, Blanchard L, et al. (2015) Inflammatory Stress on Autophagy in Peripheral Blood Mononuclear Cells from Patients with Alzheimer's Disease during 24 Months of Follow-Up. PLoS One 10: e0138326.

41. Holthoff JH, Woodling KA, Doerge DR, Burns ST, Hinson JA, et al. (2010) Resveratrol, a dietary polyphenolic phytoalexin, is a functional scavenger of peroxynitrite. Biochem Pharmacol 80: 1260-1265.