RESEARCH ARTICLE   **OPEN ACCESS**

ALZHEIMER'S ALTERS AMPK SIGNALING PATHWAY AND BIOMARKERS OF SUBCLINICAL INFLAMMATION IN LEUKOCYTOS STIMULATED WITH RESVERATROL

*Luciana C. Cardoso, Filipe N. Franco, Glaucy R. de Araújo, Barbara N.M. Silva and Miriam M. Chaves

Biochemistry Laboratory of Aging and Correlated Diseases, Department of Biochemistry and Immunology, Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte/MG – Brazil

ABSTRACT

Alzheimer's disease (AD) is considered the most prevalent form of dementia in the elderly and represents the fifth leading cause of death in this population. In addition, free radicals seem to play a fundamental role in brain aging, since several studies have found an association between cytotoxicity promoted by the accumulation of βA and oxidative stress. In this context, Resveratrol (RSV) is a polyphenol that has antioxidant and anti-inflammatory activity. However, its mechanism of action in elderly AD is still unclear. Therefore, the aim of this study was to analyze the possible modulating and/or neuroprotective effect of resveratrol from human leukocytes isolated from patients without and with AD. Our results showed that RSV has both antioxidant and anti-inflammatory activity. Among its possible mechanism of action, there is a correlation between the AMPk cell signaling pathway. However, it was possible to observe that its action occurs more accentuated in elderly people without AD, having shown a greater neuroprotective effect.

INTRODUCTION

Aging can be associated with loss of functionality, resulting in a decrease in skills, such as learning, memory and intellectual activity, due to degeneration of the central nervous system. In this context, Alzheimer's disease (AD) is considered the most prevalent form of dementia in the elderly and represents the fifth leading cause of death in this population. It is considered a neurodegenerative disorder, affecting the cortex and hypothalamus, which can cause a progressive worsening of the individual's cognitive and motor function, resulting in speech impairment, recognition of words and objects, as well as the inability to perform voluntary movements (Souza *et al* 2020). In addition, AD is characterized by the presence of senile plaques, which are formed by the deposition of β-amyloid peptides (βA) in the brain of individuals affected by the disease, in addition to presenting areas of chronic inflammation (Vingtdeux *et al*. 2010). Associated with this process, free radicals seem to play a fundamental role in brain aging, as several studies have found an association between cytotoxicity promoted by the accumulation of βA and

oxidative stress, resulting in an imbalance between the production and removal of reactive species oxygen (ROS). This increase in reactive species due to the reduction of antioxidant defenses is responsible for neurodegeneration mediated by oxidative stress (Viegas *et al*. 2011). In this context, there are antioxidants substances capable of retarding or inhibiting oxidation, defending the body from free radicals, maintaining homeostasis. Among them, the polyphenols family stands out, such as resveratrol (RSV), a stylbenoid compound found especially in plant sources such as peanuts, grapes and blackberries (Diaz-gerevini *et al*. 2016). Among the biological properties of RSV, is its ability to increase mitochondrial biogenesis (seen through its antioxidant effect), anti-inflammatory, anti-diabetic and anti-cancer. Its best known cellular mechanisms are through its ability to activate the AMPk signaling pathway. These pathways mediate different responses to stress (in particular inflammation), observed as one of the causes of aging and its related diseases (Albertoni *et al*. 2015). Thus, the aim of our study was to verify whether Alzheimer's disease alters the signaling pattern of the AMPK pathway and the biomarkers of subclinical

chronic inflammation generated by Resveratrol in donors without Alzheimer's.

METODOLOGY

Donor selection: The project was approved by the Ethics and Research Committee of the Federal University of Minas Gerais - CAAE: 14846619.7.0000.5149. Men and women over 60 years old were selected and separated into two groups: without (control group) and with Alzheimer (AD). Those who were able to participate were invited to sign the Free and Informed Consent Form and their guardians, the Free and Informed Consent Term. Detailed medical history, physical examination and laboratory data for each subject were recorded. Inclusion and exclusion criteria followed Moraes *et al*. (2010). Ten individuals of both genders were selected as subjects for the research. AD patients were selected by Dr Rafael Pacheco Terra (CRM: 45302), with the following examinations: physiological evaluation; cognitive functions; mental health (Mini Mental). Patients had a diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and related Disorders Association (NINCDS-ADRDA) criteria, and no major concomitant pathology (Oliveira *et al*. 2012). The demographic characteristics of the population are shown in Table 1.

Table 1. Demographic characteristics of the study population

Parameters	Control		Alzheimer
	Mean ± SE		Mean ± SE
Age (years)	76.3 ± 7	ns	78 ± 5.4
Body mass index (kg/m²)	23.4 ± 3	ns	25.5 ± 4.0
Serum Glucose (mg/dL)	102.6 ± 11.3	ns	105.6 ± 10.6
Serum Triglycerides (mg/dL)	164.3 ± 20	ns	153.5 ± 23
Serum creatine (mg/dL)	0.741 ± 0.17	ns	0.994 ± 0.169
Vascular Complications	No		No

ns = not significant by Mann-Whitney test. Body mass index range = 18.5–24.99 kg/m2 (Martin McKee, 2000). Serum glucose: Normal value less than 99 mg/dL (Alexandria *et al*., 2011). Triglycerides: Normal levels <150 mg/dL (American Heart Association, 2017). Serum Creatine: Normal levels 0.5–1.5 mg/dL for men and 0.6–1.2 mg/dL for women (Koch., 2000). n=10 Control group and n=10 Alzheimer group.

Obtaining leukocytes: Leukocytes were isolated according to the technique described in the literature, with adaptations (Wencel *et al*. 2017). In this way, 4 mL of heparinized blood was added in 3 mL of leucopaque gradient (density 1.12). After centrifugation (600g), two distinct phases were obtained and separated by two interphasic rings. The plasma formed corresponding to the first phase was discarded. The leukocytes were placed in another tube and resuspended with PBS (pH 7.3) through two washing sessions. Finally, the cells were suspended in 1 ml of PBS and the end the volume was adjusted to 1×10^7 cells/ml.

Padronization of H_2O_2 and resveratrol concentration: The chosen H_2O_2 concentrations were previously chosen from the literature (Emamgholipour *et al*. 2016). First, the cells were stimulated with increasing concentrations of H_2O_2 for 1 h at 37°C: 50, 150 and 250 μM. Cell viability was performed using the trypan blue assay. In addition, the chemiluminescence test was performed in order to assess which concentrations were capable of generating an oxidizing environment. The resveratrol concentration was defined based on the Dose-Response-Curve. For this, 5×10^3 cells/well were stimulated

with increasing concentrations of resveratrol: 0.63 μM; 1.25 μM; 2.5 μM; 5 μM; 10 μM and 20 μM for 24 hours at 37°C. After treatment, 100μL of resazurin (0.125 mg/L) was added to wells and the plate was incubated for 3 hours at 37°C. Absorbance was read at 570 nm in microplate reader (Thermo Plate). Results were expressed as absorbance *vs* concentration ± SE.

Cell viability assay: The MTT colorimetric assay was performed to verify cell viability against treatments with H_2O_2, RSV and H_2O_2 + RSV. For this, 5×10^3 cells / well were seeded with their respective treatments for 1 h at 37°C. After that, 20 μL of MTT solution was added to the wells and the plate was incubated for 30 min at 37°C protected from light. Finally, 100 μL of DMSO was added to dissolve the generated formazan crystals and the absorbance was read at 570 nm in a microplate reader (Thermo Plate). The results were expressed as a percentage of viability ± SE.

Chemiluminescence Assay

Part I - Production of ROS

The quantitative determination of ROS was performed through the luminol-dependent chemiluminescence assay, according to Horta *et al*. (2005) with some adaptations. First, the leukocytes (1×10^6 cell) were divided into four groups, with different treatments: (1) Negative control: leukocytes and luminol (10^{-4}M) were added in a tube; (2) Positive Control: leukocytes, H_2O_2(150 μM) and luminol were added to the tube; (3) Treatment: leukocytes, H_2O_2 (150 μM) + RSV (5 μM) and luminol were added to the tube and (4) Treatment: leukocytes, RSV (5 μM) and luminol were added to the tube. The final volume of all tubes was completed to 700 μl with PBS. Each tube was immediately placed in a Luminometer (Lumat, LB 9501, EG&G) and the reading was performed in 10 minutes. The results were expressed in units of relative light (RLU) / minute.

Part II - Evaluation of AMPk signaling pathway

The mechanism of action of resveratrol in relation to AMPk was also evaluated in a luminol-dependent chemiluminescence assay. The same assay described above was performed, but in the presence or absence of the AMPk inhibitor (Compound C - 10 μM) (Zhang *et al*. 2011). Initially, leukocytes (1×10^6 cells) were incubated with the inhibitor for 30 min at 37 °C.Then, these groups were centrifuged at 400 g for 10 min and the pellet was resuspended in PBS. The same treatments performed for groups 1, 2, 3 and 4, as described above, were added. The final volume of all tubes was completed at 700 μl with PBS and each tube was immediately placed in a luminometer for reading for 10 min. The results were expressed in units of relative light (RLU) / minute.

Dosage of cytokines IL-6, TNF and IL-10: After the leukocytes received the respective treatments for 24h at 37°C, the supernatant was collected and the procedures described in the specific kits used to measure the activity of IL-6 (Human IL-6 Uncoated ELISA- Thermo Fisher Scientific), TNF (Uncoated human TNF ELISA - Thermo Fisher Scientific) and IL-10 (Uncoated human IL-10 ELISA - Thermo Fisher Scientific) were performed. The absorbance was read at 450 nm and 570 nm in a microplate reader (Thermo Plate). The calculations were performed by analyzing the slope of the

curve and linear regression and the results were expressed as mean ± SE.

Statistical analysis

All data were analyzed using the Kolmogorov - Smirnov normality test. Univariate analysis of variance (one-way ANOVA) was used, followed by the Dunnett or Bonferroni test when the samples had a normal distribution and the Dunns test when they did not follow the normal distribution. Differences were considered significant for $p < 0.05$. All analyzes were performed using the GraphPad Prism software version 6.0 (San Diego, California, USA).

RESULTS

Initially, it was defined which H_2O_2 concentration would be used in the study. The results obtained are shown in Figure 1. In general, it was demonstrated that the cell viability of the leukocytes remained above 85% in the three concentrations analyzed. The next step was to verify if these concentrations were able to generate the same amount of ROS by chemiluminescence assay. We observed that 150 μM and 250 μM were able to induce a necessary stimulus for the production of ROS, when compared to basal cell control. There was no difference between the two highest concentrations. Therefore, the concentration chosen for the following experiments was 150μM.The resveratrol concentration was defined according to Dose-Response-Curve obtained in Figure 2.
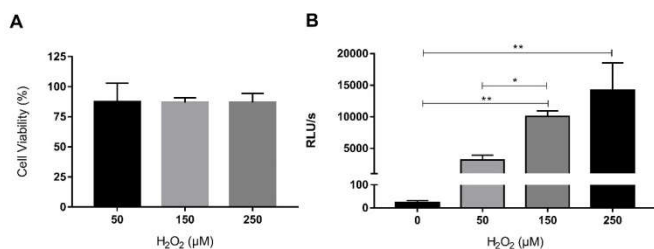

Figure 1. Padronization of H_2O_2 concentration used in the study. (A) Cells were stimulated by increasing concentrations of H_2O_2 for 1 hour (50 μM, 150 μM and 250 μM) and viability was tested by the Trypan Blue assay. (B) Evaluation of H_2O_2-induced ROS production in the Chemiluminescence assay. ROS generation was expressed in RLU/min. * p<0.05 and ** p<0.001 significant result by ANOVA analysis followed by Dunn's test (n=6)
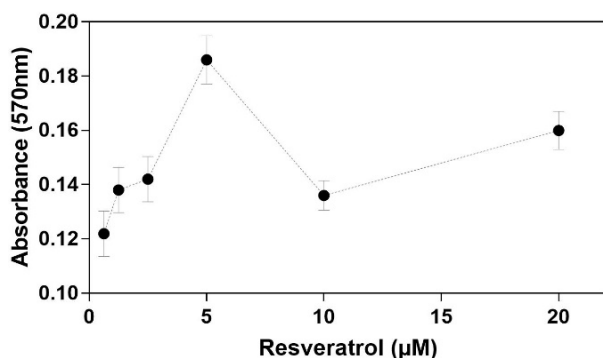

Figure 2. Dose-Response-Curve of resveratrol. Human leukocytes were stimulated by increasing concentrations of resveratrol (0.63 μM; 1.25 μM; 2.5 μM; 5 μM; 10 μM and 20 μM) and absorbance to verify stimulation in cells was measured at 570 nm. The concentration chosen was 5 μM which showed an absorbance peak and corresponds to physiological dosage. Assays were performed in triplicate for each concentration. (n = 8).

Among concentrations tested, 5μM presented a higher resazurin metabolizing capacity with a cell viability >95%. After this, it was necessary to prove that the cells remained viable under the treatment conditions. The results of the MTT assay showed that all treatments allowed the cells to remain viable and not promote significant cytotoxicity in the group with or without AD, after defining the concentrations of H_2O_2 and resveratrol (Figure 3). Analyzing the production of ROS by the chemiluminescence assay (Table 2) it is possible to observe that baseline ROS of elderly people with AD was 160% higher compared to elderly people without AD. The RSV inhibited the baseline stress of the leukocytes of the elderly without AD 30% more than the leukocytes of the elderly with AD. H_2O_2 generated cell stress in both donors without AD and with AD. RSV inhibited the effect of H_2O_2 in both donors [without AD = 1640 ± 178 (88%) and with AD = 968 ± 42 (71%)], this effect being 20% less in the elderly with AD. The results when AMPk signaling was inhibited is shown in Figure 4.
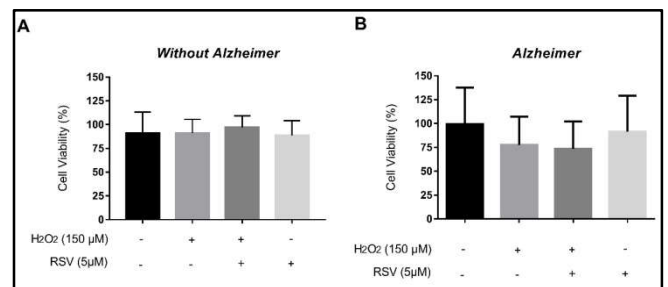

Figure 3. Evaluation of the viability of leukocytes isolated from elderly people without and withAD submitted to treatments (H_2O_2 - 150 μM and RSV - 5 μM). Viability was achieved through the MTT colorimetric assay and expressed in% of viable cells (n=6)

Table 2. Evaluation of the antioxidant effect of resveratrol on leukocytes in the chemiluminescence assay

Treatment	Without Alzheimer	Alzheimer
	Mean ± SE	Mean ± SE
Basal	437±56	1.137±114
Resveratrol (5μM)	46.25±5,89	429±67,54
H2O2 (150 μM)	13.791±1245****	3.367±456****
H2O2 (150μM)+ Resveratrol (5μM)	1.640±178 ####	968±42 ####

The generation of ROS was expressed in RLU/min. ****p<0.0001 compared to Basal group and #### compared to H_2O_2 group significant by ANOVA-Kruskal-Wallis test, Dunn's Multiple Comparison post-test (n = 8 without Alzheimer and n = 6 with Alzheimer).

The addition of Compound C increased the effect of H_2O_2 by 40% and 116% in the elderly without (19345 ± 2196) and with AD (34219 ± 3789) respectively, this increase being 190% of the elderly with AD compared to the elderly without AD. RSV was able to reverse the effect of the AMPk pathway inhibitor in both donors [without AD = 1471 ± 189 (92%) and with AD = 10334 ± 1908 (70%)], with elderly people with DA were less efficient 24%. Finally, the anti-inflammatory action of RSV in individuals with and without AD was evaluated.The results obtained in the measurement of the pro-inflammatory cytokines TNF and IL-6 show the same profile (Figure 5). There was an increase when treated only with H_2O_2 and a decrease when treated only with RSV or in the combination H_2O_2 + RSV. In addition, our results show a difference in the levels of these cytokines in the two groups. Individuals with AD have a high inflammatory profile, because in all treatment
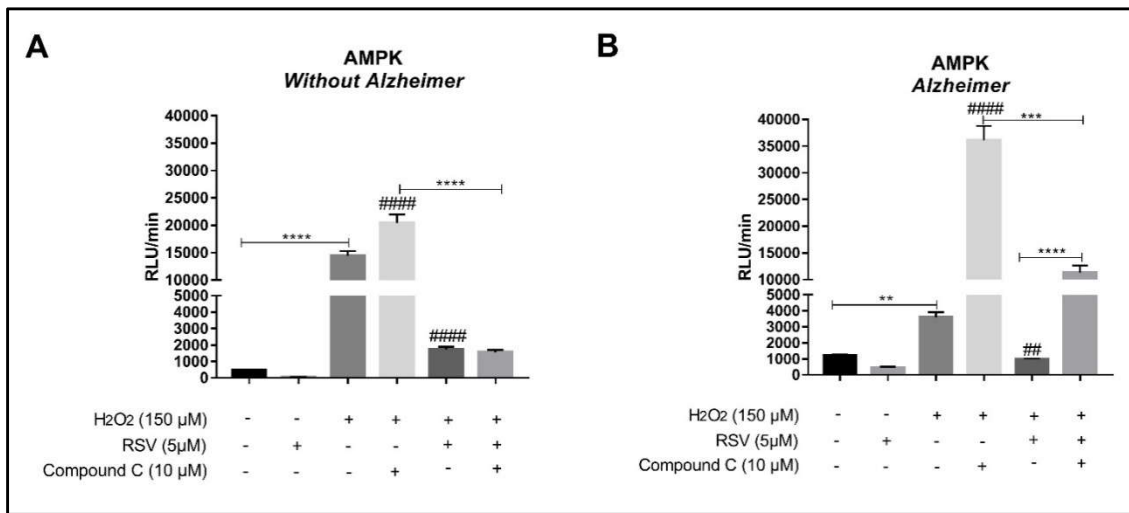
Figure 4. Evaluation of AMPk signaling pathway in leukocytes by chemiluminescence assay. Effect of resveratrol on ROS production induced by H_2O_2, being without (A) and with Alzheimer (B) in the AMPk pathway. The generation of ROS was expressed in RLU / min. * p <0.05; ** p <0.01; *** p <0.001 and **** p<0.0001 significant by ANOVA-Kruskal-Wallis test, Dunn's multiple comparison post-test. #, ##, #### vs H_2O_2 group (n = 8 without AD and n = 6 with AD)
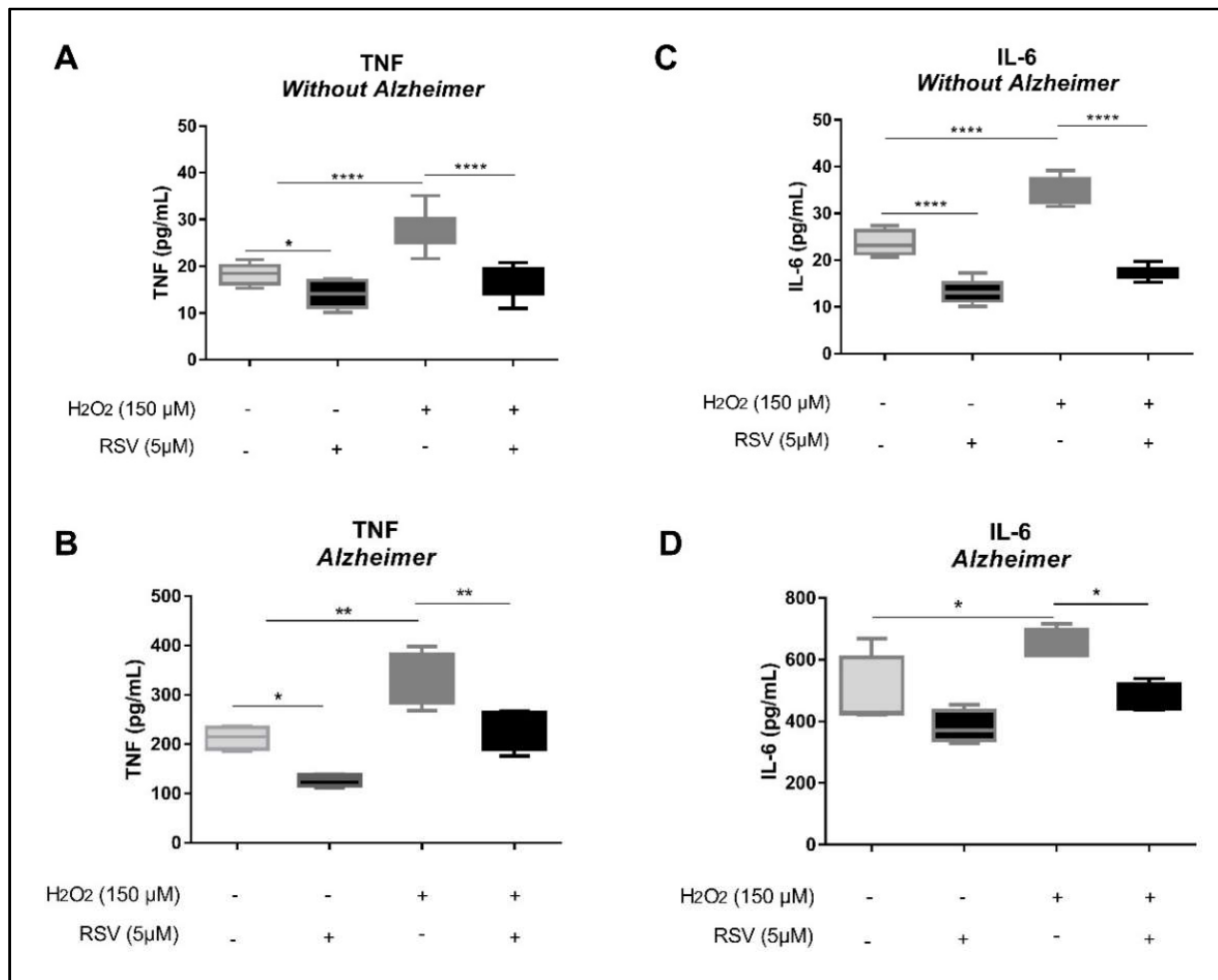

Figure 5. Effect of resveratrol on the pro-inflammatory activity of human leukocytes. TNF measurement, without (A) and with Alzheimer (B). IL-6 dosage, without (C) and with Alzheimer (D). Activity was measured in pg / mL (picograms / milliliters). * p <0.05; ** p <0.01; *** p<0.001 and **** p <0.0001 significant by the ANOVA test, post-test of the Multiple Comparison of Bonferroni (n = 7 without Alzheimer and n = 4 with Alzheimer)

conditions they have higher levels of these cytokines. The analysis of the anti-inflammatory cytokine IL-10 is shown in Figure 6. Lower levels of IL-10 are noted in all treatment conditions of individuals with AD. There was a decrease in the groups treated only with H_2O_2 and an increase in the group treated only with RSV. However, in the H_2O_2 + RSV combination, only the group without AD was able to increase the levels of this cytokine when compared to the H_2O_2 group. That is, in an oxidizing environment, resveratrol has a better anti-inflammatory potential in elderly people without AD.
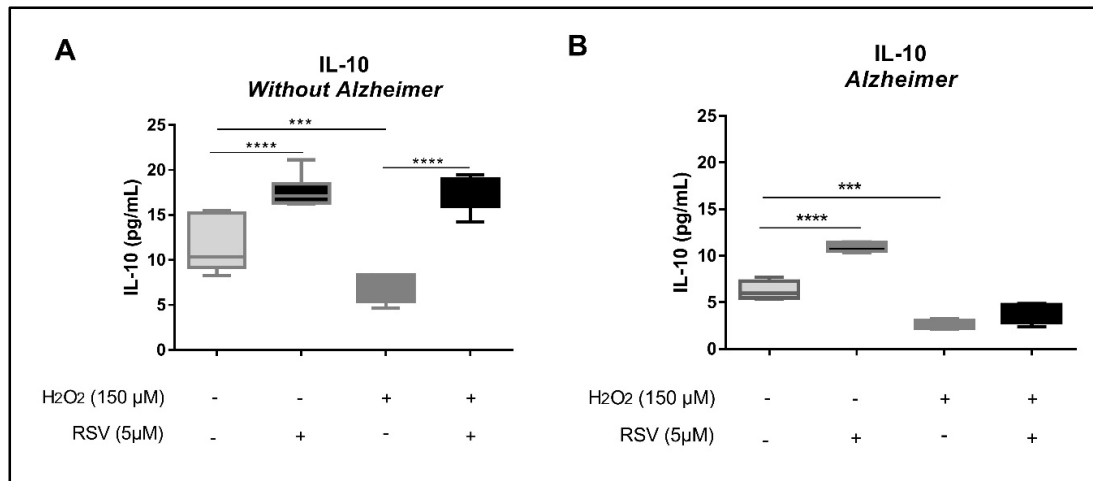
Figure 6. Effect of resveratrol on the anti-inflammatory activity of human leukocytes. IL-10 measurement, without (A) and with Alzheimer (B). Activity was measured in pg / mL (picograms / milliliters). * p <0.05; ** p <0.01; *** p<0.001 and **** p <0.0001 significant by the ANOVA test, post-test of the Multiple Comparison of Bonferroni (n = 7 without Alzheimer and n = 4 with Alzheimer)

DISCUSSION

Our study aimed to understand the mechanisms of antioxidant and anti-inflammatory action of resveratrol in elderly individuals with or without AD through possible regulation with the AMPk signaling pathway. The first step was to mimic an oxidizing environment, with the exogenous addition of H_2O_2 (150 µM) as a way to stimulate the production of reactive species (Figure 1). H_2O_2 is the main precursor of ROS, being widely used as an inducer of oxidative damage in *in vitro* studies to simulate stress conditions (Emamgholipour *et al*. 2016). In addition, the resveratrol concentration used (5 µM) was obtained on a Dose-Response Curve by our group (Figure 2) and are in agreement with several other studies in the literature (Lastra *et al*. 2007; Santos *et al*. 2021). Without significant changes in cell viability via the MTT assay (Figure 3), our chemiluminescence data showed higher baseline ROS levels in the AD group, compared to the group without AD (Table 2). When H_2O_2 was added, there was a significant induction of the oxidizing environment in both groups. ROS levels are known to be increased with aging, with significantly higher values compared to younger individuals (Luceri *et al*. 2017). In addition, mitochondrial dysfunction and increased apoptosis accompanied by a deficient antioxidant state are the mechanisms for the pathogenesis of AD. Extensive studies have pointed out the role of the superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide in neurodegeneration mediated by oxidative stress in AD (Xie *et al*. 2002; Van, 1997). Resveratrol was able to decrease the generation of ROS in both groups. These data are in accordance with the literature. In HEK-AbPP cells that received additional treatment with resveratrol, a decrease in neurotoxicity and pro-apoptotic effects was observed. The authors also discussed the importance of associating different neuroprotective agents, such as antioxidants (RSV, for example) with specific drugs for AD. Neuroprotective effects were also observed with the injection of resveratrol in rats, which reduced the accumulation of amyloid, increased expression of the antioxidant enzyme heme oxygenase-1 (HO-1) and suppression of lipid peroxidation in the hippocampus (Huang *et al*. 2011).

When we talk about oxidative stress, the evaluation of the mechanisms that modulate the activity of antioxidant compounds is important to be able to verify how this metabolic balance occurs. In this context, when studying resveratrol, one signaling pathway deserve our attention: AMPk (Figure 4). Our results showed a decrease in ROS independently of AMPk and AMPk in the group of elderly people without AD. However, the antioxidant action of resveratrol was dependent on the AMPk pathway in the AD group by inhibiting this pathway, its action was impaired. Several studies have shown that the activation of AMPk suppresses inflammation by inhibiting NF-κB, preventing oxidative stress. In this context, resveratrol has also been described as a potent activator of AMPk, implying as a pathway through which its neuroprotective effects can be exerted (Chiang *et al*. 2018). Studies have shown that resveratrol decreases the generation of ROS and increases the activity of the ERK1/2-RSK-nNOS pathway, activating AMPk to negatively regulate the NADPH oxidase induced during hypertension associated with oxidative stress. Several studies in cell and animal models have shown that resveratrol exhibits anti-inflammatory and antioxidant effects, inhibits the aggregation of beta-amyloid protein (Aβ) and modulates the intracellular effectors involved in the survival of neuronal cells. Vingtdeux *et al*. showed that resveratrol activated AMPk, increasing intracellular calcium levels and that inhibition of AMPk neutralized the effect of resveratrol on Aβ levels. This effect was also achieved in vivo, in which the peripheral administration of resveratrol activated AMPk and reduced brain levels of Aβ and accumulation in the mouse cerebral cortex (Lin *et al*. 2018).

Finally, our was to check the anti-inflammatory profile of resveratrol in the elderly without and with AD (Figure 5-6). Neuroinflammation is another important feature in AD, which manifests itself through the proliferation and activation of microglia. Microglia are macrophages residing in the brain and forming dense clusters around β-amyloid plaques. These cells function as damage trackers to the central nervous system and detect lesions in the brain parenchyma. After detecting damage or immune stimulation in the brain, such as that caused by Aβ peptides, the microglia cells are activated and produce pro-inflammatory mediators, such as, for example,

tumor necrosis factor (TNF). The accumulation of these mediators contributes to the progression of the disease (Yao *et al.* 2015). In general, our results are divided into two parts: AD group had a much more pronounced inflammatory profile than elderly without AD group. In addition, resveratrol was able to decrease the levels of the pro-inflammatory cytokines TNF and IL-6 and increase the levels of the anti-inflammatory cytokine IL-10 in both groups, the results being more expressive in the elderly without Alzheimer's. Our data are in accordance with the literature (Luigi *et al.* 2001). Capiralla *et al.*(2012), demonstrate that the administration of resveratrol in BV-2 and RAW 264.7 cells inhibited Aβ-mediated action through the activation of microglia and lipopolysaccharide (LPS). Such activation was stimulated by NF-κB, and was able to reduce the expression of its target genes, such as TNF and IL-6. When investigating the beneficial effect of resveratrol on microglia, Song *et al.* (2014) also observed the same anti-inflammatory profile as resveratrol. There was an increase in the levels of IL-10 mRNA under hypoxia conditions. In addition, resveratrol acted through the NF-κB gene, which is upstream in controlling inflammatory reactions in the microglia.

Conclusion

We concluded that resveratrol showed antioxidant and anti-inflammatory activity in both experimental groups. However, there was greater action in the group without AD, suggesting a potential preventive effect. Analyzing the cellular signaling pathway AMPk, it was possible to observe that the antioxidant capacity of resveratrol in individuals with AD decreases when this pathway is inhibited, showing the importance of this pathway in the antioxidant capacity of resveratrol during AD.

Support

Conflict of Interest: The authors declare that there is no conflict of interest.

REFERENCES

Albertoni, G. *et al*. (2015) Resveratrol and kidney disease. J Bras Nefrol. 37(1), pp. 106-114.

Alexandria, V. A. *et al*. (2011). American Diabetis Association. Standards of Medical Care in Diabetes. *Diabetes Care, 34*(Suppl 1), S11–S61.

Aronow, W. S. *et al*. (2017). American Heart Association. Heart Disease and Stroke Statistics. *Circulation, 135*(10), e146–e603.

Capiralla, H., Vingtdeux, V., Zhao, H. *et al*. (2012) Resveratrol mitigates lipopolysaccharide- and Aβ-mediated microglial inflammation by inhibiting the TLR4/NF-κB/STAT signaling cascade. J Neurochem. 120(3), pp. 461-72.

Chiang, M.C., Nicol, C.J., Cheng, Y.C. (2018) Resveratrol activation of AMPk dependent pathways is neuroprotective in human neural stem cells against

amyloid-β-induced inflammation and oxidative stress. Neurochem Int. 115, pp. 1-10.

Diaz-Gerevini, Tomas, G. *et al*. (2016) Beneficial action of resveratrol: how and why?. Nutrition. 32(2), pp. 174-178.

Emamgholipour, S., Hossein-Nezhad, A., Ansari, M. (2016) Can Melatonin Act as an Antioxidant in Hydrogen Peroxide-Induced Oxidative Stress Model in Human Peripheral Blood Mononuclear Cells? Biochem Res Int. 16, pp.585-598.

Horta, B.C., Perilo, C.S., Costa, D.C. (2005). Aging: Functional Metabolic Balance among cAMP, cGMP and Reactive Oxygen Intermediate Generation by Human Granulocytes. Gerontology. pp.363-368.

Huang, T.C., Lu, K.T., Wo, Y.Y.P., Wu, Y.J., Yang, Y.L. (2011). Resveratrol Protects Rats from Aβ-induced Neurotoxicity by the Reduction of iNOS Expression and Lipid Peroxidation. Musaro A, organizador. PLoS One. 6, pp.291-302.

Koch, W. (2000). National Committee for Clinical Laboratory Standards. Approved guideline C28-A2. How to define and determine reference intervals in the clinical laboratory. *Pennsylvania: NCCLS*.

Lastra, C. A., Villegas, I. (2007). Resveratrol as an antioxidant and pro-oxidant agent: Mechanisms and clinical implications. Biochemical Society Transaction. 35(5), pp. 1156–1160.

Lin, Y.T., Wu, Y.C., Sun, G.C. *et al*. (2018) Efeito do resveratrol no comprometimento cognitivo induzido por espécies reativas de oxigênio em ratos com doença de Alzheimer precoce induzida por angiotensina II [†]. J Clin Med. 7, pp. 329.

Luceri, C., Bigagli, E., Femia, A.P., Caderni, G., Giovannelli, L., Lodovici, M. (2017) Mudanças relacionadas ao envelhecimento em espécies reativas de oxigênio circulante (ROS) e carbonilas de proteína são indicativas de lesão oxidativa do fígado. Toxicol Rep. 5, pp. 141-145.

Luigi, A.D., Fragiacomo, C., Lucca, U. *et al*. (2001) Inflammatory markers in Alzheimer's disease and multi-infarct dementia. Mechanisms of Ageing and Development. 122, 6, pp. 1985-1995.

Martin McKee. World Health Organization. The world health report 2000: health systems: improving performance. Geneva: WHO; 2000.

Moraes, E.N., Moraes, F.L. (2010) Coleção guia de bolso em geriatria e gerontologia Incapacidade cognitiva: Abordagens diagnósticas e terapêuticas das demências no idoso. Folium Editorial. 117, pp. 117–32.

Oliveira, B.F., Veloso, C.A., Nogueira-Machado, J.A. *et al*. (2012) Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients. Nutr Neurosci. 15(6), pp. 244-51.

Santos, M.A., Franco, F.N., Caldeira, C.A., de Araújo, G.R., Vieira, A., Chaves, M.M., Lara, R.C. (2021) Antioxidant effect of Resveratrol: Change in MAPK cell signaling pathway during the aging process. Archives of Gerontology and Geriatrics. 92, pp. 1-8.

Song, J., Cheon, S.Y., Jung, W., Lee, W.T., Lee, J.E. (2014) O resveratrol induz a expressão de interleucina-10 e fator neurotrófico derivado do cérebro na microglia BV2 sob hipóxia. Int J Mol Sci. 15, pp. 15512-15529.

Sousa, J.C. *et al*. (2020) Resveratrol in Alzheimer's disease: a review. Arq Neuropsiquiatr. 78(8), pp. 501-511.

Van Dyke, K. (1997) O possível papel do peroxinitrito na doença de Alzheimer: uma hipótese simples que poderia ser testada mais profundamente. Hipóteses médicas. 48(5), pp.375–380.

Viegas, F. *et al*. (2011) Doença de Alzheimer: Caracterização, Evolução e Implicações do Processo Neuroinflamatório. Rev. Virtual Quim. 3(4), pp. 286-306.

Vingtdeux, V., Giliberto, L., Zhao, H., *et al*. (2010) A ativação da sinalização da proteína quinase ativada por AMP pelo resveratrol modula o metabolismo do peptídeo beta-amilóide. J. Biol. Chem. 285, pp. 9100–9113.

Wencel, P.L., Lukiw, W.J., Strosznajder, J.B., Strosznajder, R.P. (2018) Inhibition of Poly(ADP-ribose) Polymerase-1 Enhances Gene Expression of Selected Sirtuins and APP Cleaving Enzymes in Amyloid Beta Cytotoxicity. Mol Neurobiol. 55(6), pp. 4612-4623.

Xie, Z., Wei, M., Morgan, TE, *et al*. (2002) O peroxinitrito medeia a neurotoxicidade do beta-peptídeo amilóide 1-42- e da microglia ativada por lipopolissacarídeo. The Journal of Neuroscience. 22(9), pp. 3484–3492.

Yao, Y., Li, J., Niu, Y., Yu, J.Q., Yan, L., Miao, Z.H., *et al*. (2015) Resveratrol inhibits oligomeric Aβ-induced microglial activation via NADPH oxidase. Mol Med Rep. 12(4), pp. 6133-9.

Yeh, T.C., Shin, C.S, Chen, H.H., Lai, C.C., Sun, G.C., Tseng, C.J., Cheng, P.W. (2018) O resveratrol regula a pressão sanguínea aumentando a sinalização da AMPk para diminuir a regulação de uma NADPH oxidase derivada de Rac1 no sistema nervoso central. J. Appl. Physiol. 125, pp. 40–48.

Zhang, X., Tang, N., Hadden, T.J. *et al*. (2011) Akt, Foxo and regulation of apoptosis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 1813(11), pp. 1978-1986.

****